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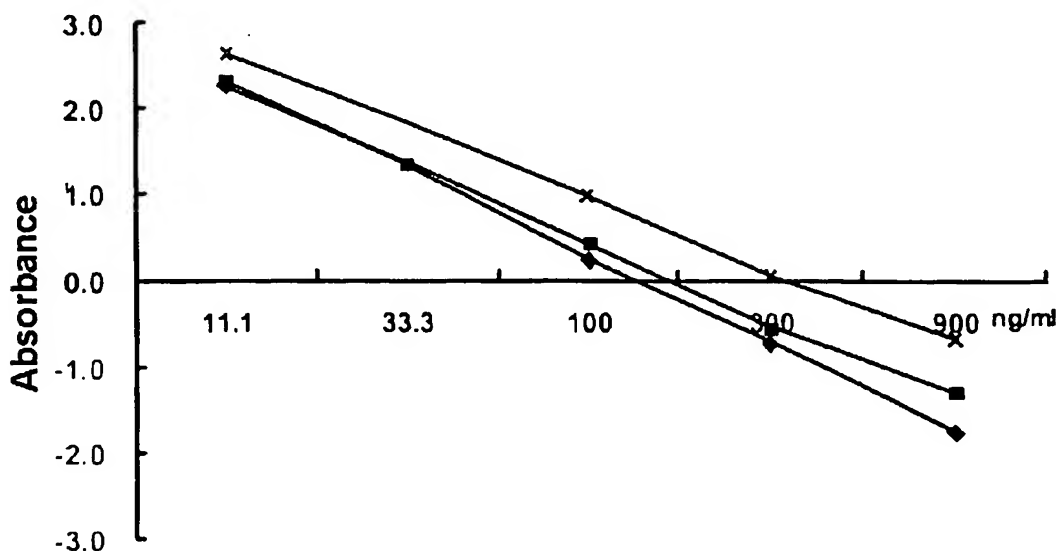
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(54) Title: THE METHOD FOR MEASURING THE AMOUNT OF β ig-h3 PROTEIN AND DIAGNOSTIC KIT USING THE SAME



(57) Abstract: The present invention relates to the method for measuring the amount of β ig-h3 protein and diagnostic kit using the same. Particularly, it relates to the method for measuring the amount of β ig-h3 protein in the body fluids by specific binding reaction between β ig-h3 protein or recombinant proteins of fas-1 domain in the β ig-h3 protein (including their fragments or their derivatives) and their ligands and relates to diagnostic kit for the renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases comprising β ig-h3 protein or recombinant proteins of fas-1 domain in the β ig-h3 protein (including their fragments or their derivatives) and their ligands. The method and kit of the present invention can be effectively used as sensitive diagnostic method for the extent of damage or progress of the renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases.

**THE METHOD FOR MEASURING THE AMOUNT OF β ig-h3
PROTEIN AND DIAGNOSTIC KIT USING THE SAME**

FIELD OF THE INVENTION

5 The present invention relates to a method for
measuring the amount of β ig-h3 protein and diagnostic
kit using the same. Particularly, it relates to a
method for measuring the amount of β ig-h3 protein in
the body fluids by specific binding reaction between β
10 ig-h3 protein or recombinant proteins of fas-1 domain
in the β ig-h3 protein (including their fragments or
their derivatives) and their ligands and relates to
diagnostic kit for the renal diseases, hepatic diseases,
rheumatoid arthritis or cardiovascular diseases
15 comprising β ig-h3 protein or recombinant proteins of
fas-1 domain in the β ig-h3 protein (including their
fragments or their derivatives) and their ligands.

BACKGROUND ART OF THE INVENTION

20 β ig-h3 is an extracellular matrix protein
induced by TGF- β in many kinds of cells including
human melanoma cells, mammary epithelial cells,
keratinocytes and lung fibroblasts. TGF- β
(transforming growth factor- β) is involved in the

growth and differentiation of many kinds of cells and the mammals have three kinds of TGF- β (TGF- β 1, TGF- β 2 and TGF- β 3). The TGF- β has been known to have many sophisticated functions such as growth control, immune response regulation, stimulating bone-formation, inducing cartilage specific macromolecule, stimulating the wounding healing, etc (Bennett, N.T. et al., *Am. J. Surg.* 1993, 165, 728). TGF- β is expressed in epithelial cells during wound-healing, probably in order to stimulate the expression of integrin in keratinocytes during the regeneration of epithelial cells. Recent studies on TGF- β expression disclosed that TGF- β 3 mRNA is expressed both in epithelia of normal skin and in epithelia under recovery from acute or chronic wounds while TGF- β 1 mRNA is expressed only in regenerated epithelia from acute wounds and TGF- β 2 mRNA is not expressed at all (Schmid, P. et al., *J. Pathol.*, 1993, 171, 191). Though the concrete theory on the mechanism of the above has not been established yet, TGF- β is believed to play a key role in regeneration of epithelia.

β ig-h3, a TGF- β induced gene h3, was first found by Stonier et al. Precisely, the β ig-h3 was found during the search of cDNA library differential screening data from A549 cell line, a human lung

adenocarcinoma cell line treated with TGF- β 1 and it was reported that β ig-h3 was 20-fold increased 2 days after TGF- β 1 treatment (Stonier, J. et al., *DNA cell Biol.*, 1992, 11, 511). It was also confirmed by DNA sequencing that β ig-h3 is composed of 683 amino acids represented by SEQ. ID. No 1 having amino-terminal secretory sequence and carboxy-terminal Arg-Gly-Asp(RGD) enabling ligand recognition against some integrins.

β ig-h3 contains 4 homogeneous internal repeated domains along with RGD motif, which are observed in membrane proteins or secretory proteins of mammals, insects, sea urchin, plants, yeasts and bacteria, etc in a state of well-preserved sequence. Proteins such as periostin, fasciclin I, sea urchin HLC-2, algal-CAM and mycobacterium MPB70 also contain the above preservative sequence (Kawamoto, T. et al., *Biochem. Biophys. Acta.*, 1998, 1395, 288). The homogeneous domain (referred as "fas-1 domain" hereinafter) preserved well in those proteins is composed of 110 - 140 amino acids containing two very preservative branches (H1 and H2) composed of 10 amino acids each. β ig-h3, periostin and fasciclin I have 4 fas-1 domains, HCL-2 has 2 and MPB70 has only 1 fas-1 domain. Some of those proteins, as cell adhesion molecules, are known to intermediate the attachment and the detachment of

cells although the biological functions of those proteins are not been fully explained yet. For example, β ig-h3, periostin and fasciclin I intervene the attachment of fibroblasts, osteoblasts and nerve cells, respectively and algal-CAM is confirmed to be a cell adhesion molecule residing in embryos of volvox (LeBaron, R. G. et al., *J. Invest. Dermatol.*, 104, 844, 1995; Horiuchi, K. et al., *J. Bone Miner. Res.*, 1999, 14, 1239; Huber, O. et al., *EMBO J.*, 1994, 13, 4212).

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A purified β ig-h3 protein stimulates adhesion and spread of fibroblasts of skin but obstructs adhesion of A549, HeLa and WI-38 cells in serum-free medium. Especially, the β ig-h3 obstructs tumor cell growth, colony formation and appearance. In fact, tumor cell growth in nude mouse prepared by transfecting Chinese hamster ovary cells with β ig-h3 expression vector was remarkably decreased, which was clearly stated in US patent #5,714,588 and #5,599,788. In addition, a method for stimulating spread and adhesion of fibroblasts around the wounded area by contacting required amount of β ig-h3 with the wound was also stated in those patents. Therefore, as a cell adhesion molecule highly induced by TGF- β in many cells, β ig-h3 plays an important role in cell growth, cell differentiation, wound healing, morphogenesis and cell

25

adhesion.

Although β ig-h3 is an effective useful material,
it is not fully supplied since only the minimum β ig-h3
5 is generated in human body. In order to solve this
problem, a method to prepare β ig-h3 by expressing it
in eukaryotic cell system using genetic engineering was
developed. In that case, though, the growth of cells
producing β ig-h3 was much slower than that of other
10 cells, resulting in difficulty in obtaining enough
amount of β ig-h3 producing cells. Therefore, the
present inventors established a purification method
with which mass-expression of recombinant proteins
containing whole β ig-h3 protein or some of its domains
15 was possible using *E.coli* as a host, confirmed that
those recombinant proteins supported cell adhesion and
spread, and applied for a patent (Korea patent
Application #2000-25664).

20 Cell adhesion activity of β ig-h3, a cell adhesion
molecule, was first reported in human dermal
fibroblasts and then disclosed in chondrocytes,
peritoneal fibroblasts and human MRC5 fibroblasts as
well. Cell adhesion activity of β ig-h3 was thought to
25 be mediated by RGD motif residing in carboxyl terminal
of β ig-h3 in the early days. But it was reported

later that RGD motif was not required for stimulating the spread of chondrocytes and a mature β ig-h3 in which RGD motif was deficient by carboxyl-terminus processing could hinder cell adhesion. Resultingly, it was confirmed that RGD motif was not an indispensable mediator for cell adhesion activity of β ig-h3. Recent studies have further confirmed that β ig-h3 stimulates cell adhesion and spread, especially the spread of fibroblasts, by working with integrin $\alpha 1\beta 1$ independently while RGD motif of β ig-h3 is not required for cell spread mediated by β ig-h3 (Ohno, S., et al., *Biochim. Biophys. Acta*, 1999, 1451, 196). Besides, H1 and H2 peptides stored in β ig-h3 have been confirmed not to affect β ig-h3-mediated cell adhesion, suggesting that certain amino acid required for cell adhesion locates not in H1 and H2 but in other sites in β ig-h3. In order to support the above, the homology between repeated fas-1 domain of β ig-h3 and fas-1 domains of other proteins was analyzed by computer, resulting in the confirmation of the fact that there were many other preservative amino acids except H1 and H2 in β ig-h3 that participated in cell adhesion.

Therefore, the present inventors tried to find out a preservative motif participating in cell adhesion and detachment activity, and to prepare a peptide containing thereof. As a result, the present inventors

have prepared peptides NKDIL, EPDIM and their derivatives mediating cell adhesion and detachment by working with a $\alpha 3\beta 1$ integrin using the second and the forth domains of β ig-h3 which is known as a cell
5 adhesion molecule and have disclosed that two very preservative amino acids, aspartic acid (Asp) and isoleucine (Ile) which are located near H2 region in the second and the forth domains of β ig-h3, are required amino acids for cell adhesion and detachment
10 activity, leading to the application for a patent (Korea Patent Application #2000-25665).

As of today, there was no report that β ig-h3 directly relates to diseases but β ig-h3 seems to be
15 related with some human cancers. The relation of β ig-h3 expression with the progress of renal diseases, hepatic diseases, rheumatoid arthritis and cardiovascular diseases has not been explained yet and the possibility to take advantage of β ig-h3 protein
20 for a diagnosis of the diseases by measuring the amount of β ig-h3 protein in body fluids has not been reported either.

Thus, the present inventors developed a method to
25 measure the amount of β ig-h3 using the recombinant protein prepared by linking many β ig-h3 or the forth

fas-1 domain of β ig-h3 together as a standard protein and a diagnostic kit using the same. The present inventors completed this invention by confirming that the method and the kit of the present invention can be effectively used as sensitive diagnostic method for the extent of damage or progress of the renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method to measure the amount of β ig-h3 protein using the β ig-h3 protein or recombinant proteins including fas-1 domains of β ig-h3 and a diagnostic kit using the same.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram showing the structure of β ig-h3 recombinant protein,

I, II, III and IV: each domain,

▣ and ▤ : base sequence preservative area

A ; β ig-h3, B ; human β ig-h3, C ; mouse β ig-h3

FIG. 2 is a diagram showing the geometrical

structure of β ig-h3 D-IV recombinant proteins prepared
by repeating β ig-h3 IV domains,

A ; β ig-h3, B ; β ig-h3 D-IV(1x),
C ; β ig-h3 D-IV(2x),
5 D ; β ig-h3 D-IV(3x), E ; β ig-h3 D-IV(4x)

FIG. 3 is an electrophoresis photograph of
separated β ig-h3 recombinant protein,

1 ; human β ig-h3, 2 ; mouse β ig-h3

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FIG. 4 is an electrophoresis photograph of β ig-h3
D-IV (1x, 2x, 3x, 4x) proteins,

1 ; β ig-h3 D-IV(1x), 2 ; β ig-h3 D-IV(2x),
3 ; β ig-h3 D-IV(3x), 4 ; β ig-h3 D-IV(4x)

15

FIG. 5 is a photograph showing the result of
Western blot using primary antibody, by which human β
ig-h3 and mouse β ig-h3 were confirmed,

1 ; human β ig-h3, 2 ; mouse β ig-h3

20

FIG. 6 is a diagram showing the principle of
enzyme-linked immunosorbent assay (ELISA),

FIG. 7 is a graph showing the quantitative ratios
25 of the primary antibody,

◆ ; 1 : 200, ■ ; 1 : 400, ▲ ; 1 : 800,

× ; 1 : 1600, ※ ; 1 : 2000, ● ; 1 : 3200

FIG. 8 is a graph showing the quantitative ratios of the secondary antibody,

- 5 A ; fixed primary antibody at 1:1600,
 B ; fixed primary antibody at 1:2000,
 ◆ ; diluted secondary antibody at 1:1000,
 ■ ; diluted secondary antibody at 1:2000,
 ● ; diluted secondary antibody at 1:3000

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FIG. 9 is a graph showing the coating concentration of human β ig-h3 protein,

◆ ; 0.5 $\mu\text{g}/\text{ml}$, ■ ; 1.0 $\mu\text{g}/\text{ml}$

15

FIG. 10 is a graph showing that both human β ig-h3 protein and mouse β ig-h3 protein can be used as standard proteins, which was confirmed by cross-test,

20 ◆ ; human β ig-h3 protein coating concentration 0.5 $\mu\text{g}/\text{ml}$, primary anti-human β ig-h3 antibody 1:2000, secondary antibody 1:2000,

 ■ ; human β ig-h3 protein coating concentration 0.5 $\mu\text{g}/\text{ml}$, primary anti-mouse β ig-h3 antibody 1:2000, secondary antibody 1:2000,

25 ▲ ; mouse β ig-h3 protein coating concentration 0.5 $\mu\text{g}/\text{ml}$, primary anti-human β ig-h3 antibody 1:2000, secondary antibody 1:2000,

× ; mouse β ig-h3 protein coating concentration
0.5 $\mu\text{g}/\text{ml}$, primary anti-mouse β ig-h3 antibody 1:2000,
secondary antibody 1:2000

5 FIG. 11 is a graph showing that recombinant β ig-h3
h3 D-IV(1x) protein and recombinant β ig-h3 D-IV(4x)
protein can be used as standard proteins, which was
confirmed by cross-test,

10 ◆ of A ; β ig-h3 D-IV(1x) coating concentration
0.5 $\mu\text{g}/\text{ml}$, primary anti-human β ig-h3 antibody 1:2000,
secondary antibody 1:2000,

 ■ of A ; β ig-h3 D-IV(4x) coating concentration
0.5 $\mu\text{g}/\text{ml}$, primary anti-human β ig-h3 antibody 1:2000,
secondary antibody 1:2000,

15 ◆ of B ; β ig-h3 D-IV(1x) coating concentration
0.5 $\mu\text{g}/\text{ml}$, primary anti-mouse β ig-h3 antibody 1:2000,
secondary antibody 1:2000,

20 ■ of B ; β ig-h3 D-IV(4x) coating concentration
0.5 $\mu\text{g}/\text{ml}$, primary anti-mouse β ig-h3 antibody 1:2000,
secondary antibody 1:2000

FIG. 12 is a photograph of an
immunohistochemical-staining showing the expression
pattern of β ig-h3 in renal tissue,

25 ► of A ; expression pattern at basal membrane of
S3 proximal tubular cell,

► of B ; expression pattern at basal membrane of
Bowman's capsule of glomerulus,

→ of B ; expression pattern at basal membrane of
cortical thick ascending limb cell

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FIG. 13 is a graph showing the levels of β ig-h3
in urine of diabetes-induced rats,

■ ; control group,

□ ; diabetes-induced rats by treatment of
10 streptozotocin

FIG. 14 is a graph showing the individual level
of β ig-h3 in urine of diabetes-induced rats of FIG. 13,

15

FIG. 15 is a graph showing the level of β ig-h3 in
urine obtained from each a normal rat, a rat with
nephron underdose, a rat with chronic rejection, a rat
with recurrent GN and a rat showed CyA toxicity,

20

FIG. 16 is a graph showing the different
concentrations of β ig-h3 protein by the day that were
measured with urine samples of patients who have been
under the treatment of plasmapheresis since focal
segmental glomerulosclerosis (FSGS) was re-developed
25 after kidney transplantation,

FIG. 17 is a graph showing the concentrations of β ig-h3 protein in urine taken from a living donor, cadaver donor, a patient with underdose and rejection that were measured before and after kidney transplantation,

FIG. 18 is a photograph of an immunohistochemical-staining showing the expression pattern of β ig-h3 protein in the injured blood vessels of diabetes-induced mouse,

A ; normal blood vessels,

B ; injured blood vessels, L ; lumen

FIG. 19 is a graph showing the expression pattern of β ig-h3 protein in the culture of vascular smooth muscle cells,

* ; $p < 0.05$,

** ; $p < 0.01$

DETAILED DESCRIPTION OF THE INVENTION

To achieve the above object, the present invention provides a method for measuring the amount of β ig-h3 protein.

The present invention also provides a diagnostic kit for the renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases using

the same.

Further features of the present invention will appear hereinafter.

5 The method for measuring the amount of β ig-h3 of the present invention consists of following steps:

1) Preparing β ig-h3 protein or recombinant proteins containing β ig-h3 fas-1 domain, their fragments or derivatives;

10 2) Preparing specific ligands against the above recombinant proteins, their fragments or derivatives of the above step 1; and

15 3) Measuring the amount of β ig-h3 protein of samples with the method using binding reaction of ligands of the above step 2 with the recombinant proteins, their fragments or derivatives of the above step 1.

20 In the step 1, β ig-h3 protein is either a human β ig-h3 protein having amino acid sequence represented by SEQ. ID. No 3 or a mouse β ig-h3 protein having amino acid sequence represented by SEQ. ID. No 5. The structural elements of human and mouse β ig-h3 proteins are shown in FIG. 1. Hatched region and cross-hatched region of FIG. 1 show very well preserved sequences of
25 repeated fas-1 domain I, II, III and IV and blank

region represents RGD motif.

β ig-h3 protein has 4 fas-1 domains. For the β ig-h3 fas-1 domain of the above step 1, it is preferable to select one or more than two out of the first through the 4th fas-1 domain of β ig-h3 protein and is more preferable to use the 4th fas-1 domain. The 4th fas-1 domain could be used either individually or as a recombinant protein in which many fas-1 domains are repeatedly linked. For the recombinant protein, 1 to 10 fas-1 domains are required to be combined and using 1 to 4 fas-1 domains is more preferred. In the preferred embodiments of the present invention, the present inventors provided examples of using the 4th fas-1 domain only and recombinant proteins prepared by linking two, three and 4 forth fas-1 domains of β ig-h3 respectively.

The present inventors prepared proteins each represented by SEQ. ID. No 7, No 8, No 9 and No 10 having one of the 4th fas-1 domains containing 502nd - 632nd amino acids of β ig-h3, two, three and four of those respectively and named them " β ig-h3 D-IV(1x)", " β ig-h3 D-IV(2x)", " β ig-h3 D-IV(3x)" and " β ig-h3 D-IV(4x)" (see FIG. 4).

Epitope of β ig-h3 protein at which specific binding reaction with ligand is occurring and any other part of the protein containing peptides hydrolyzed by

protease can be used as fragments of the recombinant protein. Derivatives of the recombinant protein of the present invention can be prepared by covalent bond including phosphorylation or glycosylation, and non-
5 covalent bond including ionic bond, coordinate bond, hydrogen bond, hydrophobic bond or van der Waals' bond. If fragments of the derivatives of the above recombinant proteins could be specifically bound to ligands, they would be included in the category of the
10 proteins of the present invention.

For the preparation of the standard protein of the present invention, the construction of expression vector and the transformation could be performed by the conventional method.

15

In the step 2, ligands that are specifically binding to β ig-h3, β ig-h3 fas-1 domain, fragments or derivatives thereof can be confirmed by observing the binding reaction of ligands with the protein or
20 recombinant protein of the step 1. There are many kinds of ligands such as antibody, RNA, DNA, organic compounds including lipid, protein or organic salts, or inorganic compounds including metal ions or inorganic salts, and preferable ligand is a primary antibody
25 against β ig-h3 or β ig-h3 fas-1 domain of the step 2 made by using the protein or the recombinant protein

(fragments or derivatives included) of the step 1 as an antigen. The primary antibody can be prepared by the conventional method and monoclonal antibody or polyclonal antibody can be used.

5

In the step 3, the amount of β ig-h3 protein included in sample was measured using the specific binding reaction of ligand with β ig-h3 protein, its fragments or derivatives. Where ligand-binding reaction is occurring, even pieces of those fragments or derivatives can be used. Quantification assay using antigen-antibody binding reaction in which β ig-h3 protein is used as an antigen is preferably used. It is more preferable to select one way from a group consisting of immunoblotting (*Current Protocols in Molecular Biology*, vol 2, chapter 10.8; David et al., *Cells (a Laboratory manual)*, vol 1, chapter 73), immunoprecipitation (*Current Protocols in Molecular Biology*, vol 2, chapter 10.16; *Cells(a Laboratory manual)*, vol 1, chapter 72), ELISA (*Current Protocols in Molecular Biology*, vol 2, chapter 11.2; *ELISA Theory and Practice*, John R. Crowther; *The ELISA Guidebook*, John R. Crowther), RIA (Radioimmuno assay) (*Nuklearmedizin* 1986 Aug ;25(4):125-127, Tumor markers as target substances in the radioimmunologic detection of malignancies. von Kleist S; Mariani G. Ann

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Oncol 1999 ;10 Suppl 4:37-40), protein chip (Daniel Figeys et.al, *Electrophoresis* 2001, 22, 208-216; Albala JS. *Expert Rev Mol Diagn* 2001 Jul;1 (2):145-152), rapid assay (Kasahara Y and Ashihara Y, *Clinica Chimica Acta* 5 267 (1997), 87-102; Korea Patent Application #2000-46639) or microarray (Vivian G. cheung et al, *Nature genetics* 1999, 21, 15-19; Robert J. Lipshutz et al, *Nature genetics* 1999, 21, 20-24; Christine Debouck and Peter N. Goodfellow, *Nature genetics* 1999, 21, 48-50; 10 *DNA Microarrays*, M. Schena), and ELISA is the most preferable method. Mass-analysis of samples is also possible using biological microchip and automatic microarray system along with ELISA, and simple self-diagnostic method using urine can be developed 15 therefrom.

According to the preferable embodiments of the present invention, the method for measuring the amount of β ig-h3 protein with competition assay using ELISA 20 comprises the following steps;

- 1) Coating β ig-h3 protein or recombinant protein containing β ig-h3 fas-1 domain, its fragments or derivatives to matrix;
- 2) Reacting antibody against the protein of the 25 above step 1, its fragments or derivatives with sample;
- 3) Adding the reactant of the above step 2 to the

coated protein of step 1 and waiting for reaction, and then washing thereof; and

4) Adding the secondary antibody to the reactant of the above step 3 for further reaction, and then measuring OD.

All kinds of matrix commonly used are good for the matrix of the above step 1 and especially, nitrocellulose membrane, polyvinyl plate (for example; 96 well plate), polystyrene plate and glass slide can be used as a matrix.

The secondary antibody of the above step 4 is labeled with coloring enzymes, fluorescent materials, luminous materials, radioisotopes or metal chelates. Every commonly used labeling materials are available for this invention and peroxidase, alkaline phosphatase, β -D-galactosidase, malate dehydrogenase, staphylococcus nuclease, horseradish peroxidase, catalase and acetylcholine esterase are preferable coloring enzymes. As for fluorescent materials, fluorescein isothiocyanate, phycobilin protein, rhodamine, phycoerythrin, phycocyanin, orthophthalic aldehyde, etc are preferably used.

As another labeling materials for the secondary antibody in addition to coloring enzymes or fluorescent materials, luminous materials such as isoluminol,

lucigenin, luminol, acridiniumester, imidasol, acridine salt, luciferin, luciferase and aequorin or radioisotopes such as ^{125}I , ^{127}I , ^{131}I , ^{14}C , ^3H , ^{32}P and ^{35}S are preferably used. Besides, micromolecular heptenes
5 like biotine, dinitrophenyl, pyridoxil or fluoressamine can be also conjugated with antibody.

In the case of using coloring enzymes in step 4, coloring substrates should be used to measure the activity of the enzyme and every material that are able
10 to develop color of the enzyme bound to the secondary antibody can be used as a coloring substrate. 4-chloro-1-naphtol (4CN), Diaminobenzidine (DAB), Aminoethyl carbazole (AEC), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), o-
15 Phenylenediamine (OPD) and Tetramethyl Benzidine (TMB) are preferably used as coloring substrates.

As for the samples of the above step 2, all kinds of body fluids of patients suffering from β ig-h3 related diseases can be used. Especially, urines,
20 bloods or synovial fluids of patients suffering from renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases are preferable.

In order to confirm whether the method for
25 measuring the amount of β ig-h3 protein of the present invention is correct, the present inventors used

recombinant protein containing mouse β ig-h3 or the 4th fas-1 domain of β ig-h3 as a standard protein and compared the result with that from using human β ig-h3 as a standard protein.

5 The optimum coating concentration of human β ig-h3 protein and the quantitative ratio of antibody were determined for the method for measuring β ig-h3 of the present invention. The best quantitative ratio of the primary anti-human β ig-h3 antibody was 1:1600 and
10 1:2000 (see FIG. 7), and the best quantitative ratio of the secondary antibody was 1:2000 (see FIG. 8). The proper concentration of human β ig-h3 protein was 1.0 μ g/ml and 0.5 μ g/ml, but 0.5 μ g/ml was more preferable as coating concentration (see FIG. 9).

15 Therefore, the present inventors decided the optimum coating concentration of human β ig-h3 standard protein to be 0.5 μ g/ml and the best diluting ratio of the primary anti-human β ig-h3 antibody and the secondary antibody to be 1:2000, respectively.

20 The present inventors also determined protein concentration and the quantitative ratio of the primary antibody and the secondary antibody using mouse β ig-h3, recombinant β ig-h3 D-IV(1x), ig-h3 D-IV(2x), ig-h3 D-
25 IV(3x) and β ig-h3 D-IV(4x). Precisely, made coating concentration of each protein at 0.5 μ g/ml, diluted the

primary anti-human β ig-h3 antibody and the secondary antibody at 1:2000 respectively and performed quantitative assay. Diluted the primary anti-mouse β ig-h3 antibody and the secondary antibody at 1:2000, and performed quantitative assay as well.

As a result, graphs with straight line were made for all the cases, suggesting the ratios were the best and the measuring range of them was between 11 ng/ml - 900 ng/ml, meaning there was not much difference in the measuring range among them all (see FIG. 11 and FIG. 12).

From the above results, it was confirmed that standard protein could be any of human β ig-h3, mouse β ig-h3, recombinant β ig-h3 D-IV(1x), ig-h3 D-IV(2x), ig-h3 D-IV(3x) and β ig-h3 D-IV(4x), and either anti-human β ig-h3 antibody or anti-mouse β ig-h3 antibody could be used as the primary antibody.

In this invention, the preferable coating concentration of standard protein is 0.1 - 2.0 μ g/ml and 0.5 - 1.0 μ g/ml is more preferable. The preferable diluting ratio of the primary and the secondary antibody is 1:400 - 1:3200 and 1:2000 is more preferable.

The present invention provides a diagnostic kit for renal diseases, hepatic diseases, rheumatoid

arthritis or cardiovascular diseases, with which the diseases are diagnosed by measuring the amount of β ig-h3 protein in the body fluids of patients.

The diagnostic kit of the present invention includes β ig-h3 protein or recombinant proteins of fas-1 domain in the β ig-h3 protein (including their fragments or their derivatives) and their ligands. At this time, as preferable specific ligands, antibodies against β ig-h3 protein or β ig-h3 fas-1 domains are used. The kit can additionally include buffer solution, secondary antibody, washing solution or coloring substrate.

The diagnostic kit of the present invention is available for the diagnosis of various diseases such as renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases by measuring the amount of β ig-h3 protein in the body fluids.

It is possible to diagnose renal diseases by measuring the amount of β ig-h3 protein on the basis of the fact that β ig-h3 expression is induced by TGF- β that plays an important role in the development of renal diseases. For the confirmation of the above, measured the amount of β ig-h3 in urine of diabetic patients. As a result, the amount of β ig-h3 in urine of patients with diabetic renal diseases including

microalbuminuria was about five-fold higher than that of normal person. Some diabetic patients without renal diseases also showed higher β ig-h3 amount than normal. Considering the above result, β ig-h3 level in urine
5 seems to reflect the extent of renal damage and high β ig-h3 level of some diabetic patients without renal diseases suggests that their kidneys are already damaged to some degree, though not showing any clinical troubles yet. Therefore, measuring the amount of β ig-h3 in patients' urine is a highly sensitive and
10 important diagnostic method that can reflect the damage of kidneys in the early stage.

In order to confirm whether the β ig-h3 concentration in a diabetic patient's urine can reflect
15 the damage of a kidney in the early stage, measured the β ig-h3 concentration of a diabetic animal. As a result, the β ig-h3 concentration was 4-fold increased 5 days after inducing diabetes (see FIG. 13). Observed the changes of β ig-h3 concentration in each individual
20 after inducing diabetes, resulting in the great increase of β ig-h3 concentration in urine after inducing diabetes (see FIG. 14). On the 5th day after inducing diabetes, blood urea and creatine were normal and kidney tissues seemed normal. Thus, the great
25 increase of β ig-h3 amount in urine on the fifth day suggests that there was the minimum damage in kidney

already, which could not be detected by the traditional test methods.

The present inventors further confirmed the relation between kidney damage and β ig-h3 concentration by measuring β ig-h3 amount in urine of preoperative and postoperative patients with kidney transplantation. As a result, the high β ig-h3 concentration of a preoperative patient dropped gradually after successful operation. But in the case of No. 5 patient whose kidney function was not recovered even after operation, the β ig-h3 concentration was still great (see FIG. 2). Considering all the above results, it is for sure that the β ig-h3 concentration sensitively reflects the extent of kidney damage.

The present inventors also measured the β ig-h3 concentration in urine of renal failure patients. As a result, all of those renal failure patients showed great β ig-h3 concentration in their urine. Thus, it was confirmed again that β ig-h3 amount in urine reflects kidney damage sensitively even in the early stage, so that measuring the β ig-h3 amount is very important diagnostic method for various renal diseases (see Table 3).

Determining if a chronic hepatitis patient is

developing to a hepatocirrhosis patient is very important but there is no way to catch that so far. The most crucial factor for the development of hepatocirrhosis is TGF- β . Thus, β ig-h3 whose expression is induced by TGF- β could be possibly increased in blood as hepatocirrhosis goes on. If so, the amount of β ig-h3 can also reflect the extent of hepatocirrhosis. In fact, β ig-h3 expression was confirmed to be greater as hepatocirrhosis became serious by immunohistological test with liver tissues of hepatitis patients. The present inventors subdivided patient's condition into several grades and stages based on the biopsy results of chronic hepatitis patients and investigated blood β ig-h3 concentration of each stage and grade. Chronic hepatitis patients showed higher blood β ig-h3 concentration than normal people. β ig-h3 concentration of lower stage and grade was confirmed to be higher than that of higher stage and grade (see Table 5). Condition of a patient in grade 3 and stage 3 is that hepatocirrhosis has been developed seriously and its activity went through the peak already. Meanwhile, a patient in grade 1 and 2 and stage 1 and 2 shows the condition that inflammatory reaction is developing very actively. Thus, β ig-h3 concentration implies the activity of hepatocirrhosis, so that the development of hepatocirrhosis can be

observed by measuring blood β ig-h3 concentration regularly.

5 β ig-h3 concentration in synovial fluid of rheumatoid arthritis patients and osteoarthritis patients was also measured. As a result, two-fold higher β ig-h3 concentration in synovial fluid of rheumatoid arthritis patients was observed, suggesting that measuring β ig-h3 concentration in synovial fluid
10 can be an effective way to diagnose osteoarthritis and rheumatoid arthritis (see Table 6).

In addition, the expression patterns of β ig-h3 in normal and damaged blood vessels of diabetic mice were
15 investigated by immunohistochemical methods in order to confirm the relation between the expression of β ig-h3 and vascular diseases. As a result, β ig-h3 protein was expressed much greatly in damaged blood vessels of diabetic mice than in normal blood vessels (see FIG.
20 18). Based on that β ig-h3 expression is induced by TGF- β that plays an important role in the development of vascular diseases, TGF- β 1 inducing β ig-h3 expression in vascular smooth muscle cells forming blood vessels was investigated. As a result, it was
25 confirmed that β ig-h3 expression increases as the amount of TGF- β 1 increases (see FIG. 19).

The expression of β ig-h3 in blood and tissues reflects the damage of them. Thus, it was confirmed that the method for measuring the amount of β ig-h3 protein of the present invention can be effectively
5 used for the diagnosis of various vascular diseases.

Therefore, the diagnostic kit measuring the amount of β ig-h3 protein of the present invention is very effective in use since it reflects the extent of
10 damage and progress of renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases.

EXAMPLES

Practical and presently preferred embodiments of
15 the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the
20 spirit and scope of the present invention.

Example 1: Preparation of standard proteins and primary antibodies

<1-1> Separation of human β ig-h3 and mouse β ig-h3

25 The present inventors have prepared human and

mouse β ig-h3 proteins. The structural elements of human and mouse β ig-h3 proteins are shown in FIG. 1. Hatched region and cross-hatched region of FIG. 1 show very well preserved sequences of repeated fas-1 domain I, II, III and IV and blank region represents RGD motif.

β ig-h3 cDNA (pBS β ig-h3; obtained by cloning cDNA of human skin papilloma cells) having a base sequence represented by SEQ. ID. No 2 cloned in pBluescript SK (-) vector was digested with *Nde* I and *Bgl* II, resulting in the preparation of DNA fragments having blunt ends. The above DNA fragments were subcloned into *EcoR* V and *EcoR* I sites of pET-29 β vector (purchased from Novagen). The protein having a amino acid sequence of 69 - 653 amino acids of β ig-h3 represented by SEQ. ID. No 3 was separated and named human β ig-h3.

Next, β ig-h3 cDNA was digested with *BamH* I and *Xho* I, resulting in the preparation of DNA fragments having a base sequence represented by SEQ. ID. No 4. The above DNA fragments were subcloned into *BamH* I and *Xho* I sites of pET-29 β vector. The protein having a amino acid sequence of 23 - 641 amino acids of β ig-h3 represented by SEQ. ID. No 5 was separated and named mouse β ig-h3.

In order to express the above human and mouse β ig-h3 proteins, *E.coli* BL21(DE3) cells were transformed. The transformants were cultured in LB medium containing kanamidine (50 μ g/ml) at 37°C until their OD₅₉₅ was
5 reached to 0.5 - 0.6. During the culture, the expression of β ig-h3 protein was induced by treating 1 mM isopropyl- β -D-(-)thiogalactopyranoside (IPTG) at 37°C for 3 hours.

10 Pellets of *E.coli* cells were resuspended in cell lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethane sulfonyl fluoride (referred as "PMSF" hereinafter) and 0.5 mM DTT), and then crushed by ultrasonification. The
15 procedure was repeated 5 times.

The above solution was centrifuged and the insoluble inclusion bodies containing β ig-h3 were dissolved in 20 mM Tris-HCl buffer solution containing 0.5 M NaCl, 5 mM imidazol and 8 M urea. The proteins
20 were purified by using Ni-NTA resin (Qiagen). The proteins were dialyzed one after another in 20 mM Tris-Cl buffer solution containing 50 mM NaCl with urea starting from high concentration to low concentration for the purification and the results were confirmed by
25 SDS-PAGE.

As a result, it was confirmed that the human β ig-

h3 and the mouse β ig-h3 proteins of the present invention were purified (FIG. 2).

<1-2> Construction and separation of β ig-h3 D-IV(1x)
5 and β ig-h3 D-IV(4x)

The DNA fragment represented by SEQ. ID. No 6 encoding the 4th domain that corresponds to 498th - 637th amino acids of human β ig-h3 represented by SEQ. ID. No 1 was amplified by PCR. The PCR product was
10 cloned into pET-29 β vector to construct the expression vector of the 4th domain. The present inventors named the expression vector of the 4th domain " β ig-h3 D-IV".

Base sequence that corresponds to the 4th domain
15 was synthesized by PCR, and the 3' end of the PCR product was blunted by using klenow fragment. This PCR product was inserted into *EcoR* V site of the above expression vector p β ig-h3 D-IV, and named p β ig-h3 D-IV(2x). Inserted fragment of p β ig-h3 D-IV(2x) was
20 digested with *EcoR* V and *Xho* I, and the 3' end of the fragment was blunted by using klenow fragment. This fragment was inserted into *EcoR* V site of p β ig-h3 D-IV, and named p β ig-h3 D-IV(3x). The fragment having blunted 3' end was also inserted into *EcoR* V site of
25 p β ig-h3 D-IV(2x), and named p β ig-h3 D-IV(4x) (FIG. 3). His-tag was made by linking 6 histidine residues to

carboxyl terminal of the DNA fragment to purify proteins with Ni-NTA resin (Qiagen).

E.coli BS21(DE3) cells were transformed with the expression vectors. The transformants were cultured in LB medium containing kanamycin (50 $\mu\text{g}/\text{ml}$). Pellets of *E.coli* cells were resuspended in cell lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethane sulfonyl fluoride (referred as "PMSF" hereinafter) and 0.5 mM DTT), and then crushed by ultrasonification. The procedure was repeated 5 times. The above solution was centrifuged to obtain supernatants. The proteins were purified by using Ni-NTA resin (Qiagen) from the supernatants, and confirmed with SDS-PAGE.

As a result, it was confirmed that β ig-h3 D-IV(1x) having an amino acid sequence represented by SEQ. ID. No 7, β ig-h3 D-IV(2x) having an amino acid sequence represented by SEQ. ID. No 8, β ig-h3 D-IV(3x) having an amino acid sequence represented by SEQ. ID. No 9 and β ig-h3 D-IV(4x) having an amino acid sequence represented by SEQ. ID. No 10 proteins were expressed. All the above proteins contained the 4th domain of human β ig-h3 (FIG. 4).

<1-3> Preparation and separation of primary antibody

The primary antibody was prepared by using human β ig-h3 and mouse β ig-h3 proteins separated in Example <1-1> as an antigen. The proteins were subcutaneously injected on the back of rabbits. For the first injection, 200 μ g of proteins were mixed with complete Freund's adjuvant and then injected. For the 2nd to 5th injection, 100 μ g of proteins were mixed with incomplete Freund's adjuvant and then injected at 3-week intervals. Venous blood was collected and left at room temperature for 2 hours. Following centrifugation (10,000 \times g, 10 minutes), the supernatants containing the primary antibody were obtained. The supernatants were kept at -20°C for further usage (FIG. 5).

15 Example 2 Determination of coating concentration of human β ig-h3 protein and quantitative ratio of antibody

<2-1> Determination of quantitative ratio of the primary antibody

20 In order to determine the quantitative ratio of the primary antibody to human β ig-h3 protein, the human β ig-h3 was diluted (0.5 μ g/ml) with 20 mM carbonate-bicarbonate solution (pH 9.6, 0.02% sodium azide contained). The β ig-h3 solution was added in each well of 96-well plate (200 μ l/well) and coated

25

thereof at 4°C for overnight. The primary anti-human β ig-h3 antibody was serially diluted with diluting solution (saline-phosphate buffer solution/Tween 80) at 1:200, 1:400, 1:800, 1:1600, 1:2000 and 1:3200, and added into the coated 96-well plate. The secondary antibody (1:5000) was also added thereto and reacted thereof at room temperature for 1 and half hours. Substrate solution (prepared by dissolving o-phenylendiamine in methanol (10 mg/ml), diluting with distilled water at 1:100, and mixing with 10 μ l of 30% hydrogen peroxide solution) was also added thereto and reacted thereof at room temperature for 1 hour. The reaction was terminated by adding 50 μ l of 8 N sulfuric acid solution, and ELISA was performed (O.D 492 nm).

As a result, it was confirmed that the best quantitative ratio of the primary anti-human β ig-h3 antibody was 1:1600 and 1:2000 (FIG. 7)

<2-2> Determination of quantitative ratio of secondary antibody

In order to determine the quantitative ratio of the secondary antibody, the human β ig-h3 protein was coated on the plate (0.5 μ g/ml). Added the primary anti-human β ig-h3 antibody thereto (1:1600 and 1:2000). Added the secondary antibody thereto (1:1000, 1:2000 and 1:3000 respectively) and reacted thereof. ELISA was

performed with the same method as the above Example <2-1>.

As a result, it was confirmed that the best quantitative ratio of the secondary antibody was 1:2000 (FIG. 8).

<2-3> Determination of coating concentration of human β ig-h3 protein

In order to determine the coating concentration of human β ig-h3 protein, the primary anti-human β ig-h3 antibody was diluted at 1:2000, the secondary antibody was diluted at 1:2000, the human β ig-h3 protein was coated on the plate at 0.5 $\mu\text{g}/\text{ml}$ and 1.0 $\mu\text{g}/\text{ml}$ respectively, and then ELISA was performed.

As a result, it was confirmed that the proper concentration of human β ig-h3 protein was both 1.0 $\mu\text{g}/\text{ml}$ and 0.5 $\mu\text{g}/\text{ml}$, but 0.5 $\mu\text{g}/\text{ml}$ was more preferable as coating concentration since R^2 value approaches 1 best with that concentration (FIG. 9).

From the above results, the present inventors decided the optimum coating concentration of human β ig-h3 standard protein to be 0.5 $\mu\text{g}/\text{ml}$ and the best diluting ratio of the primary anti-human β ig-h3 antibody and the secondary antibody to be 1:2000, respectively.

The values obtained from the above result were

log transformed by Robard formula (Robard, 1971)
represented by the below <Mathematical Formula 1>.
Resultingly, a line was formed from 11 ng/ml to 900 ng/
ml, which was the possible range in measurement. It
5 was also confirmed that measurement was possible even
to the range of 10 ng/ml with the above reaction
condition (FIG. 10).

<Mathematical Formula 1>

10 $\log b = \log e^{b/(100-b)}$

In the above formula, b represents the percentage
to OD of the well that does not include any antigen in
each concentration.

15 Example 3: Measurement of quantitative range of mouse
 β ig-h3, recombinant β ig-h3 D-IV(1x) and β ig-h3 D-
IV(4x) by cross-test

The present inventors also determined protein
concentration and the quantitative ratio of the primary
20 and the secondary antibody using mouse β ig-h3,
recombinant β ig-h3 D-IV(1x) and β ig-h3 D-IV(4x).
Particularly, made coating concentration of each
protein 0.5 μ g/ml and the quantitative ratio of the
primary anti-human β ig-h3 antibody and the secondary
25 antibody to be 1:2000 for the experiments. Regulated

the quantitative ratio of the primary anti-mouse β ig-h3 antibody and the secondary antibody to be 1:2000 as well.

5 As a result, graphs with straight line were made for all the cases, suggesting the ratio was the best and the ranges of them were between 11 ng/ml and 900 ng/ml, meaning there were not much differences in the range of measurement (FIG. 11 and FIG. 12).

10 From the above results, it was confirmed that standard protein could be any of human β ig-h3, mouse β ig-h3, recombinant β ig-h3 D-IV(1x) and β ig-h3 D-IV(4x), and either anti-human β ig-h3 antibody or anti-mouse β ig-h3 antibody could be used as the primary antibody.

15 Example 4: Relationship between renal diseases and β ig-h3 expression

<4-1> Measurement of β ig-h3 in diabetics

20 The present inventors have confirmed the relationship between renal diseases and β ig-h3 expression on the basis of the fact that β ig-h3 expression is induced by TGF- β that plays an important role in the development of renal diseases. For the confirmation, measured the amount of β ig-h3 in urine of diabetics. Particularly, mixed 110 μ l of urine of
25 diabetic and 110 μ l of the primary antibody (1:1000) in

a round-bottomed plate, and cultured thereof at 37°C for 1 hour. Added 200 μl of the above mixture to β ig-h3-coated plate and reacted thereof at room temperature for 30 minutes. Stopped the reaction by adding
 5 secondary antibody-substrate stop solution, and performed ELISA (O.D 492 nm).

<Table 1>

Concentration of β ig-h3 in diabetics' urine

Samples	β ig-h3 (ng/ml)
Normal	31.0 (n=93, \pm 8.6)
Type II DM	101.9 (n=51, \pm 17.1)
Type II DM + microalbuminuria	127.4 (n=30, \pm 27.7)
Type II DM + overt proteinuria	105.4 (n=19, \pm 14.9)
Type II DM + CRF	153.6 (n=93, \pm 28.1)

10

As a result, the amount of β ig-h3 in urine of diabetic renal disease patients including microalbuminuria was about five-fold higher than that of normal. Some diabetic patients without renal
 15 diseases also showed higher β ig-h3 amount than normal. Considering the above results, β ig-h3 level in urine seems to reflect the extent of renal damage and high β ig-h3 level of some diabetic patients without renal diseases suggests that their kidneys have already been
 20 damaged to some degree, though not showing any clinical

troubles yet. Therefore, measuring the amount of β ig-h3 in patients' urine is a highly sensitive and important diagnostic method that can reflect the damage of kidneys in the early stage.

5

<4-2> Measurement of β ig-h3 in diabetic animal model

In order to confirm whether the β ig-h3 concentration in diabetic's urine can reflect the renal damage in the early stage, the present inventors measured the β ig-h3 amount of diabetic animals.

10

Diabetes was induced in Sprague-Dawley (SD) rats by injecting streptozotosin (60 mg/kg), a kind of diabetes-inducing drug, into the peritoneal cavity of the rats. Confirmed that diabetes was induced by measuring the blood-glucose of the rats. Taken urines from the rats on the fifth day after inducing diabetes, and measured the β ig-h3 amount with the same method of Example <4-1>

15

As a result, the β ig-h3 amount was 4-fold increased 5 days after inducing diabetes (56.9 ± 6.4 ng/creatinine mg : 230.4 ± 131.8 ng/creatinine mg, FIG. 13). Observed the change of β ig-h3 amount in each individual after inducing diabetes, resulting in the great increase of β ig-h3 amount in urine after inducing diabetes (FIG. 14). On the fifth day after

20
25

inducing diabetes, blood urea and creatine were normal and renal tissues seemed normal. Thus, the great increase of β ig-h3 amount in urine on the fifth day after inducing diabetes suggested that there was the minimum damage in kidney already, which could not be detected by the conventional methods.

<4-3> Measurement of β ig-h3 in patients operated on kidney transplantation

The present inventors confirmed the correlation between renal damage and β ig-h3 amount by measuring β ig-h3 amount in urines of patients before and after kidney transplantation. The results were presented in Table 2.

<Table 2>

Changes of β ig-h3 concentration in patients before and after kidney transplantation

Day / Patients	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	Success or not
1				376.9	199.2	105.6	59.1	67.6	84.5	63.1	61.2	39.7	9.9	O
2		149.2	147.3	133.5	159.5	148.3	147.3	96.0	74.0	40.7	20.3	27.9	26.4	O
3	107.8	95.8	101.4	102.3	102.2	106.1	106.6	125.5	83.5	49.4	36.5	33.3	23.2	O
4							298.8	208.1	140.5	169.9	188.4	76.3	24.4	O
5							188.6	160.7	469.3	290.9	494.7	324.4	-	X

As a result, the high β ig-h3 amount of pre-operative patients dropped gradually after successful operation. But in the case of No 5 patient whose renal function was not recovered even after kidney transplantation, the β ig-h3 amount was still great. Considering all the above results, it is for sure that the amount of β ig-h3 sensitively reflects the extent of kidney damage.

10 <4-4> Measurement of β ig-h3 in patients with renal failure

The present inventors measured the β ig-h3 amount in urines of patients with renal failure. As a result, all of those patients showed great β ig-h3 amount in their urines (Table 3).

<Table 3>

Concentrations of β ig-h3 in urines of patients with renal failure

20

Samples	β ig-h3 (ng/mg)
Normal	31.0 (n=93, \pm 8.6)
Chronic renal failure	335.4 (n=9, \pm 56.0)

<4-5> Measurement of β ig-h3 in patients with kidney related diseases

In order to investigate whether β ig-h3 was differently expressed in patients with renal diseases, the present inventors measured the β ig-h3 concentration in urines taken from patients who showed normal signs after kidney transplantation, patients whose transplanted kidney was smaller, patients who showed chronic rejection, patients with re-developed pyelitis and patients who had cyclosporine toxicity with the same method of Example <4-1>.

As a result, patients with normal signs after kidney transplantation showed 39.4 ng/creatinine mg of β ig-h3 concentration at average while patients with chronic rejection, re-developed pyelitis and cyclosporine toxicity showed greatly increased β ig-h3 concentration (140.8, 175.4 and 90.9 ng/creatinine mg, respectively) (FIG. 15, Table 4).

<Table 4>

β ig-h3	Normal after kidney transplan- tation (n=47)	Transpla- nted with small kidney (n=16)	Chronic rejection n (n=15)	Pyelitis re- develope d (n=6)	Cyclosp h orine toxicity (n=6)
---------------	---	--	-------------------------------------	---	--

Average	39.4± 18.2	54.7± 23.0	140.8± 81.1	175.4± 65.8	90.9± 22.4
Minimum	9.4	17.9	48.8	83.2	64.6
Maximum	84.7	100.0	374.4	249.8	119.4

The present inventors also investigated if the increased β ig-h3 concentration in patients with re-developed renal diseases was decreased again as treatment worked. As a result, urine β ig-h3 concentration of patients who had blood plasma exchange to treat re-developed pyelitis after kidney transplantation was gradually decreased, suggesting urine β ig-h3 concentration decreased while treatment was working. Thus, β ig-h3 concentration could be used as a marker of treatment reaction (FIG. 16).

<4-6> Analysis of effects of kidney transplantation on β ig-h3 concentration

In order to investigate the changes of urine β ig-h3 concentration after kidney transplantation, the present inventors measured urine β ig-h3 concentration of patients who had kidney transplantation everyday.

As a result, urine β ig-h3 concentration of patients who had kidney transplantation successfully, regardless the kidney was given from a living person or

a brain death person, was decreased gradually. Precisely, as for receiving kidney from a living person, urine β ig-h3 concentration came back to normal level within 4-5 days after transplantation and as for
5 receiving kidney from a brain death person, β ig-h3 concentration came back to normal level within 6-7 days (FIG. 17).

Besides, urine β ig-h3 concentration of patients who received small kidney came back to normal level
10 after transplantation though their blood creatine values were still high, suggesting that the transplanted kidney worked normal although it could not filtrate waste products well enough because of its small size. Anyway, β ig-h3 concentration reflecting
15 the damage of kidney was back to normal (FIG. 17). Meanwhile, urine β ig-h3 concentration of patients who had unsuccessful kidney transplantation fluctuated seriously.

Based on those results, urine β ig-h3
20 concentration could be used as an effective marker for diagnosis of renal diseases in the early stages, for detecting progression of renal diseases and for determination of treatment effect since β ig-h3 concentration reflects the damage of kidney well.

25 Resultingly, the present inventors confirmed that urine β ig-h3 concentration reflects the damage of

kidney in the early stages sensitively and is important and useful for diagnosis of various renal diseases.

Example 5: Relationship between hepatic diseases and
5 β ig-h3 expression

Determining if a chronic hepatitis patient is developing to a hepatocirrhosis patient is very important but there is no way to catch that so far. The most crucial factor for the development of
10 hepatocirrhosis is TGF- β . Thus, β ig-h3 whose expression is induced by TGF- β could be possibly increased in blood as hepatocirrhosis goes on. If so, the amount of β ig-h3 can also reflect the extent of hepatocirrhosis. In fact, β ig-h3 expression was
15 confirmed to be greater as hepatocirrhosis became serious by immunohistologic test with liver tissues of hepatitis patients. The present inventors subdivided patient's condition into several grades and stages based on the biopsy results of chronic hepatitis
20 patients and investigated blood β ig-h3 concentration of each stage and grade. Particularly, the present inventors collected blood from chronic hepatitis patients and measured the amount of β ig-h3 with the same method of Example <4-1>. The results were
25 presented in Table 5.

<Table 5>

Concentrations of β ig-h3 in blood of chronic hepatitis patients

5

Grade	β ig-h3 (ng/mg)	Stage	β ig-h3 (ng/mg)
0 (Normal)	146.2 (n=172, \pm 28.5)	0 (Normal)	146.2 (n=172, \pm 28.5)
1	196.6 (n=16, \pm 30.6)	1	193.4 (n=20, \pm 30.2)
2	190.0 (n=43, \pm 72.8)	2	192.2 (n=36, \pm 79.1)
3	167.5 (n=7, \pm 21.9)	3	172.5 (n=10, \pm 21.9)

As a result, chronic hepatitis patients showed higher blood β ig-h3 concentration than normal people and β ig-h3 concentration of lower stage and grade (1 and 2) was confirmed to be higher than that of higher stage and grade (3). Condition of a patient in grade 3 and stage 3 is that hepatocirrhosis has been developed seriously and its activity went through the peak already. Meanwhile, a patient in grade 1 and 2 and stage 1 and 2 shows the condition that inflammatory reaction is developing very actively. Thus, β ig-h3 concentration implies the activity of hepatocirrhosis, so that the development of hepatocirrhosis can be observed by measuring blood β ig-h3 concentration

regularly.

Example 6: Relationship between rheumatoid arthritis
and β ig-h3 expression

5 The present inventors confirmed the correlation
between rheumatoid arthritis and β ig-h3 expression by
measuring β ig-h3 amount in synovial fluids of patients
with osteoarthritis and rheumatoid arthritis with the
same method of Example <4-1> (Table 6).

10

<Table 6>

Concentrations of β ig-h3 in synovial fluids

	β ig-h3 (ng/mg)
Osteoarthritis	11.0 (n=29, \pm 0.3)
Rheumatoid arthritis	21.0 (n=20, \pm 2.5)

15 As a result, two-fold higher β ig-h3 concentration
in synovial fluid of rheumatoid arthritis patients was
observed, suggesting that measuring β ig-h3
concentration in synovial fluid can be an effective way
to diagnose osteoarthritis and rheumatoid arthritis.

20

Example 7: Relationship between cardiovascular diseases
and β ig-h3 expression

<7-1> Measurement of β ig-h3 in damaged blood vessels of diabetes-induced mice

5 The present inventors investigated the expression patterns of β ig-h3 in normal and damaged blood vessels of diabetic mice by immunohistochemical methods in order to confirm the relation between the expression of β ig-h3 and cardiovascular diseases.

10

As a result, β ig-h3 protein was expressed much greatly in damaged blood vessels of diabetic mice than in normal blood vessels (FIG. 18).

15 <7-2> Measurement of β ig-h3 expression induced by TGF- β in vascular smooth muscle cells

Based on that β ig-h3 expression is induced by TGF- β that plays an important role in the development of vascular diseases, the present inventors tried to confirm the correlation β ig-h3 expression and cardiovascular diseases. Particularly, the present inventors measured the expression pattern of β ig-h3 induced by TGF- β 1 in vascular smooth muscle cells forming blood vessels with the same method of Example

25 <4-1>.

As a result, it was confirmed that β ig-h3 expression increases as the amount of TGF- β 1 increases (FIG. 19).

5 From the above results, it was confirmed that the expression of β ig-h3 in blood and tissues reflects the damage of them. Therefore, the method for measuring the amount of β ig-h3 protein of the present invention can be effectively used for the diagnosis of various
10 cardiovascular diseases.

INDUSTRIAL APPLICABILITY

As described hereinbefore, the method for measuring the amount of β ig-h3 protein of the present
15 invention in which human β ig-h3, mouse β ig-h3, β ig-h3 D-IV(1x) or β ig-h3 D-IV(4x) are used as a standard protein is inexpensive and very accurate in measuring β ig-h3 concentration in various body fluids. The amount of β ig-h3 sensitively reflects TGF- β related
20 diseases such as renal diseases, hepatic diseases, rheumatoid arthritis and cardiovascular diseases in the early stages, so that the method of the present invention can be effectively used for the examination of the damage and the progress of those diseases and
25 for the diagnosis thereof.

What is Claimed is

1. A method for measuring the amount of β ig-h3 protein comprises the following steps:

- 5 1) Preparing recombinant proteins of β ig-h3 or β ig-h3 fas-1 domain, their fragments or derivatives;
- 2) Preparing specific ligands against the above recombinant proteins, their fragments or derivatives of the above step 1; and
- 10 3) Measuring the amount of β ig-h3 protein of samples with the method using binding reaction of ligands of the above step 2 with the recombinant proteins, their fragments or derivatives of the above step 1.
- 15

2. The method for measuring the amount of β ig-h3 protein as set forth in claim 1, wherein the ligands of step 1) are selected from a group consisting of antibodies, RNA, DNA, lipids, proteins, organic compounds and inorganic compounds.

20

3. The method for measuring the amount of β ig-h3 protein as set forth in claim 1, wherein the specific binding reaction of step 3) is antigen-

25

antibody reaction.

4. The method for measuring the amount of β ig-h3 protein as set forth in claim 3, wherein the antigen-antibody reaction is performed by a method selected from a group consisting of immunoblotting, immunoprecipitation, ELISA, RIA, protein chip, rapid assay and microarray.

5. The method for measuring the amount of β ig-h3 protein as set forth in claim 3, wherein the antigen-antibody reaction of step 3) comprises the following steps:

1) Coating recombinant protein prepared from β ig-h3 protein or β ig-h3 fas-1 domain, its fragments or derivatives to matrix;

2) Reacting antibody against the protein of the above step 1, its fragments or derivatives with sample;

3) Adding the reactant of the above step 2 to the coated protein of step 1 and waiting for reaction, and then washing thereof; and

4) Adding the secondary antibody to the reactant of the above step 3 for further reaction, and then measuring OD.

6. The method for measuring the amount of β ig-h3 protein as set forth in anyone of claim 1-5, wherein the β ig-h3 protein is human β ig-h3 protein having amino acid sequence represented by SEQ. ID. NO 3 or mouse β ig-h3 protein having amino acid sequence represented by SEQ. ID. No 5.
7. The method for measuring the amount of β ig-h3 protein as set forth in anyone of claim 1-5, wherein 1 or 2-10 4th fas-1 domains of β ig-h3 protein are repeatedly linked.
8. The method for measuring the amount of β ig-h3 protein as set forth in claim 7, wherein the fas-1 domain of β ig-h3 is selected from a group consisting of sequences represented by SEQ. ID. No 7, No 8, No 9 and No 10.
9. The method for measuring the amount of β ig-h3 protein as set forth in claim 1, wherein the sample can be any body fluid including urine, blood or synovial fluid.
10. A diagnostic kit for the renal diseases, hepatic diseases, rheumatoid arthritis or cardiovascular diseases comprising β ig-h3 protein or recombinant

proteins of fas-1 domain in the β ig-h3 protein
(including their fragments or their derivatives)
and their ligands.

5 11.The diagnostic kit as set forth in claim 10,
 wherein the ligand is selected from a group
 consisting of antibody specifically binding to β
 ig-h3 protein, fas-1 domain of β ig-h3, their
 fragments or derivatives, RNA, DNA, lipids,
10 proteins, organic compounds and inorganic
 compounds.

 12.The diagnostic kit as set forth in claim 11,
 wherein the ligand is antibody.

15 13.The diagnostic kit as set forth in claim 12,
 wherein the kit additionally includes buffer
 solution, secondary antibody, washing solution,
 stop solution or coloring substrate.

20 14.The diagnostic kit as set forth in claim 10,
 wherein the β ig-h3 protein is human β ig-h3
 protein having amino acid sequence represented by
 SEQ. ID. No 3 or mouse β ig-h3 protein having
25 amino acid sequence represented by SEQ. ID. No 5.

15.The diagnostic kit as set forth in claim 10,
wherein 1 or 2-10 4th fas-1 domains of β ig-h3
protein are repeatedly linked.

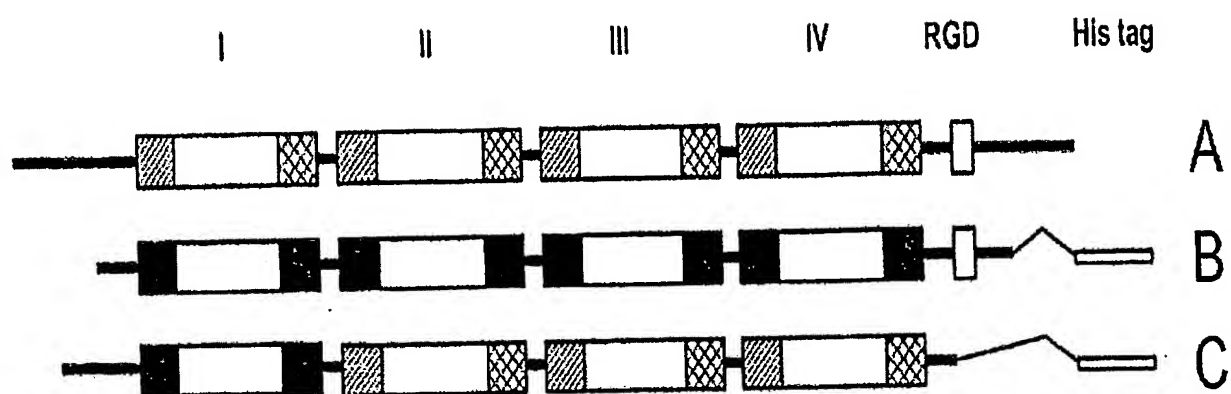
5 16.The diagnostic kit as set forth in claim 15,
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from a group consisting of sequences represented
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10

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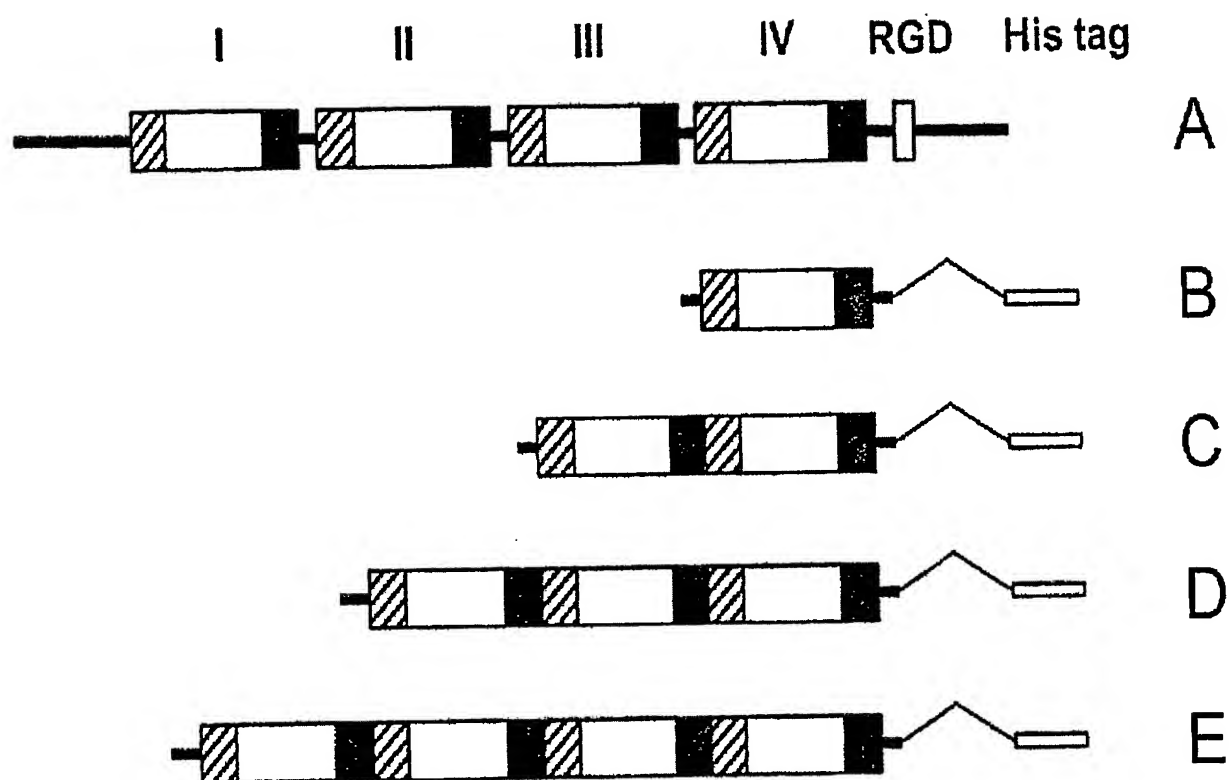
FIGURES

FIG. 1



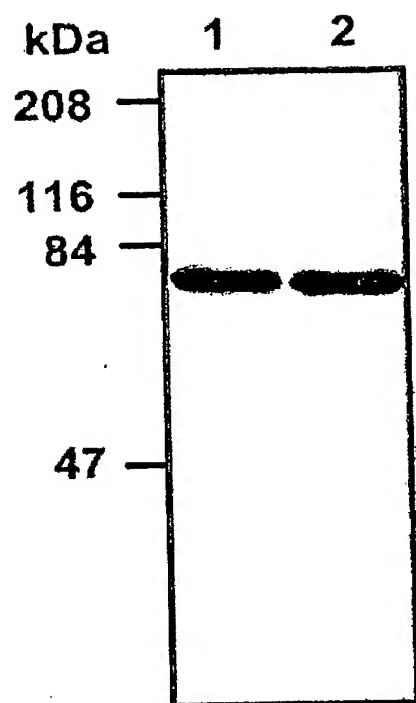
2/19

FIG. 2



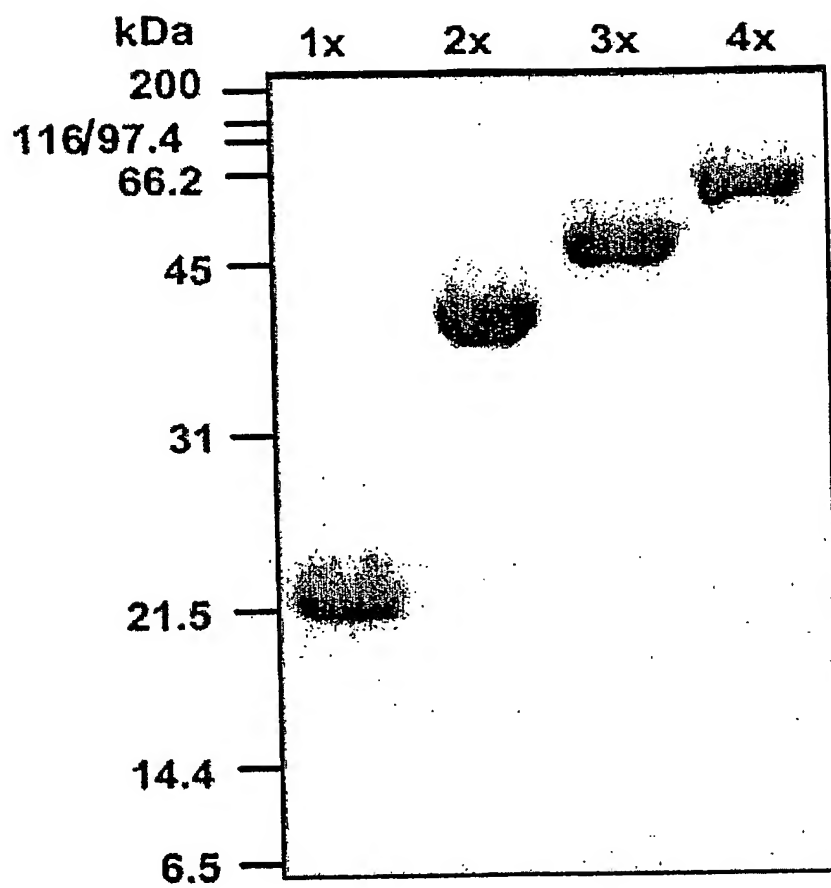
3/19

FIG. 3



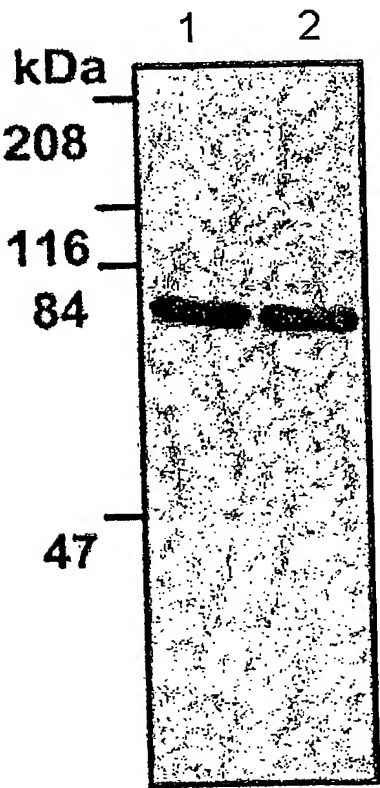
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FIG. 4



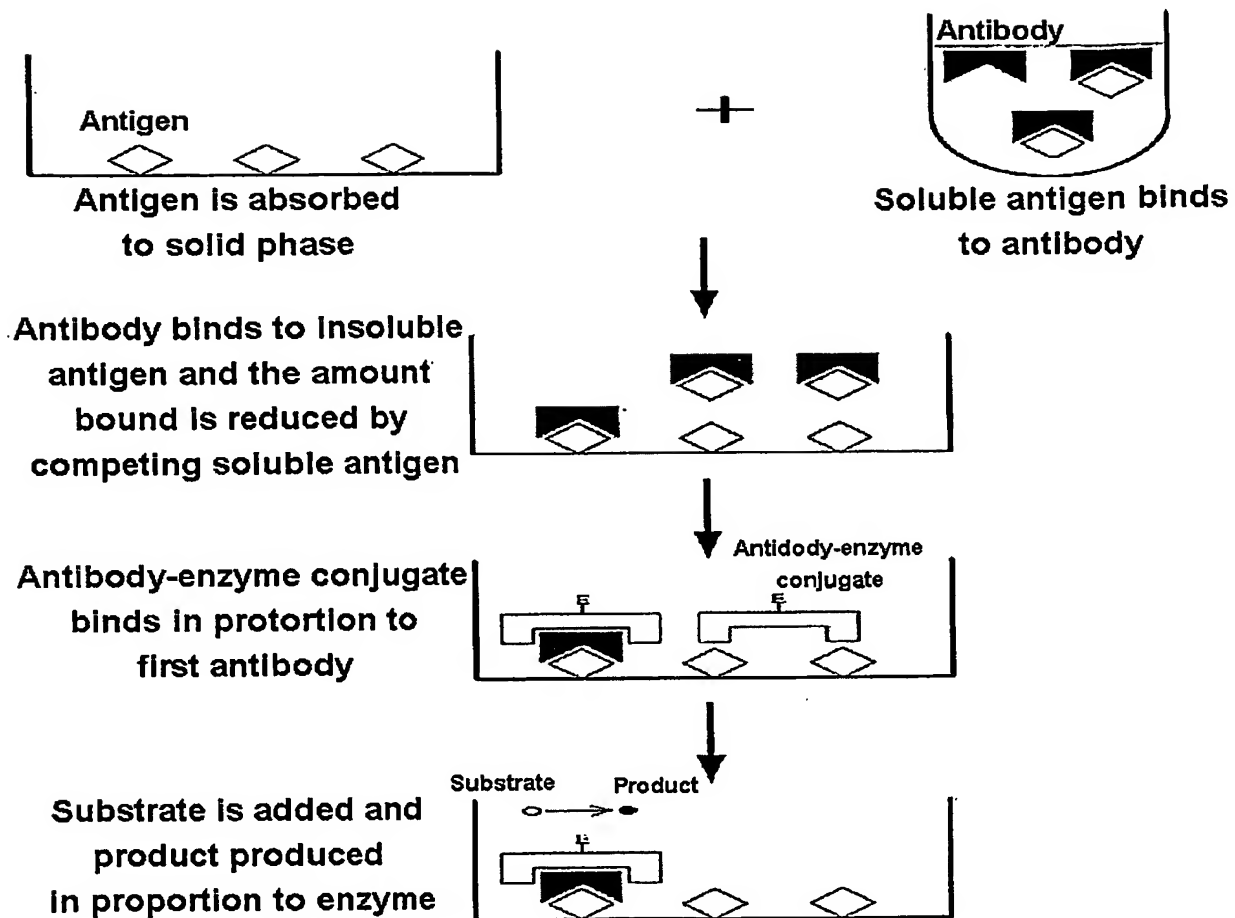
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FIG. 5



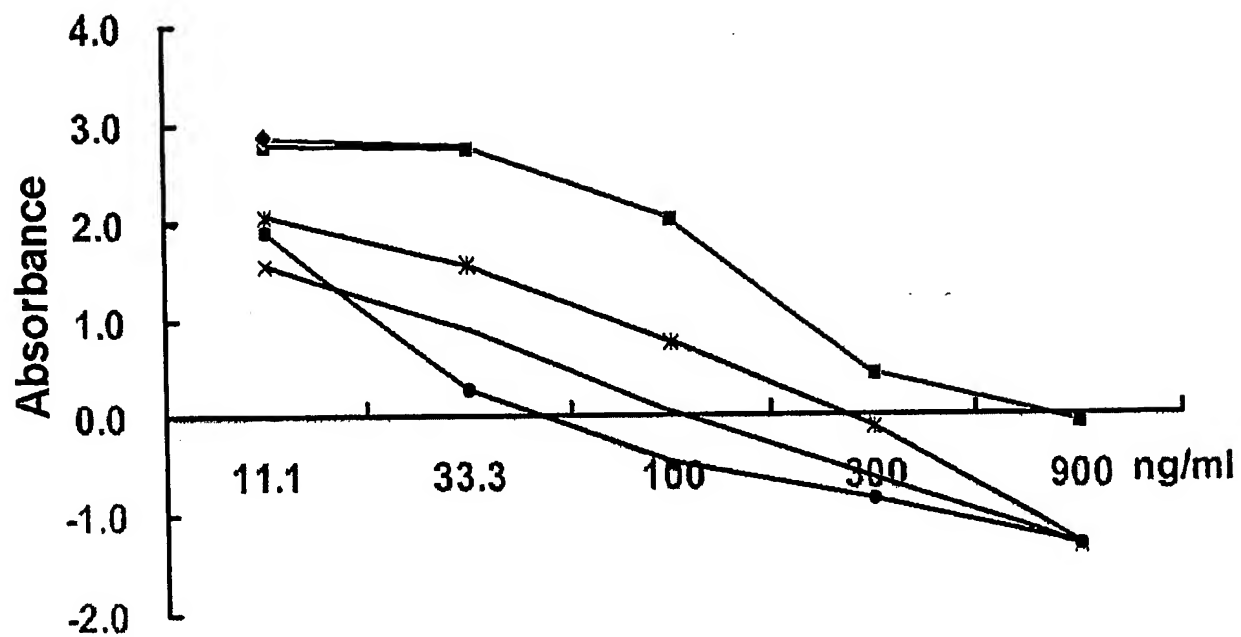
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FIG. 6



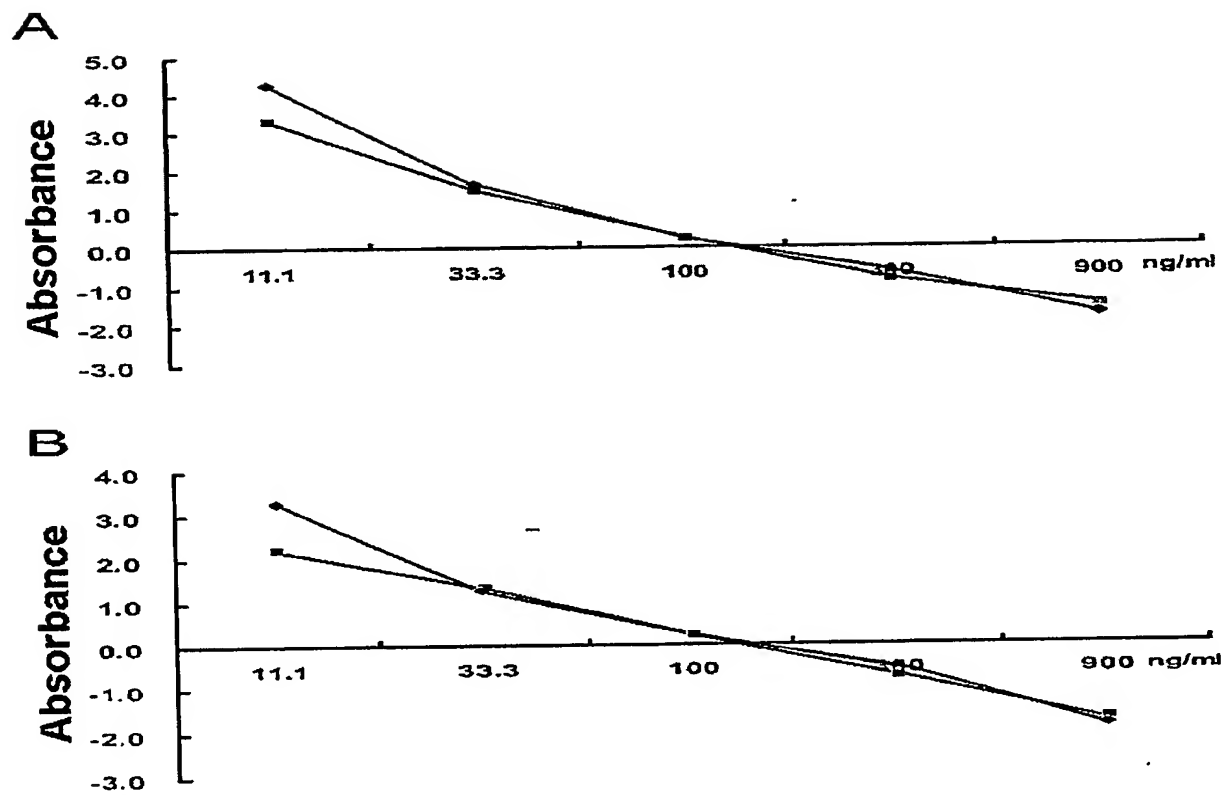
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FIG. 7



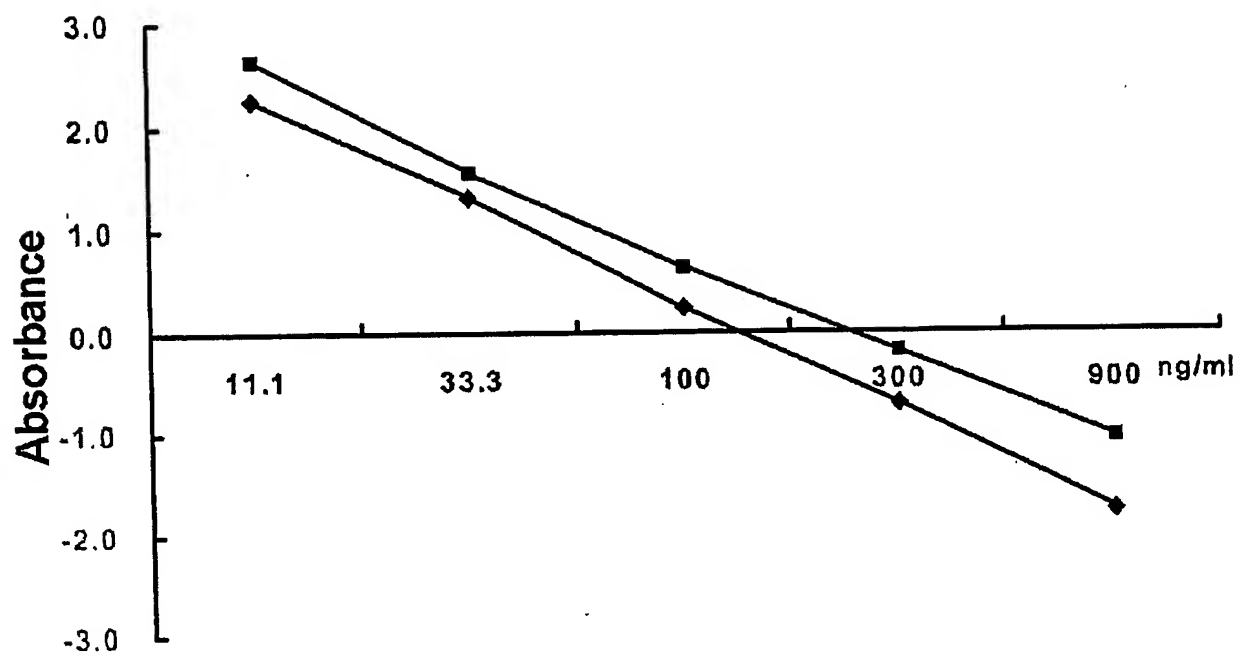
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FIG. 8



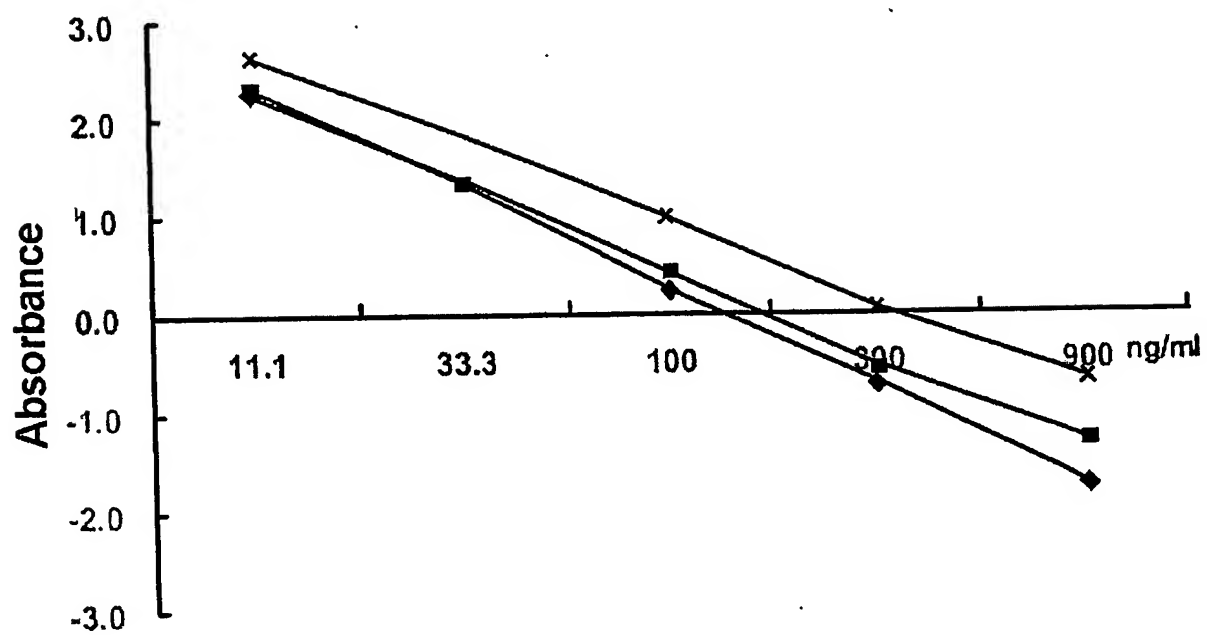
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FIG. 9

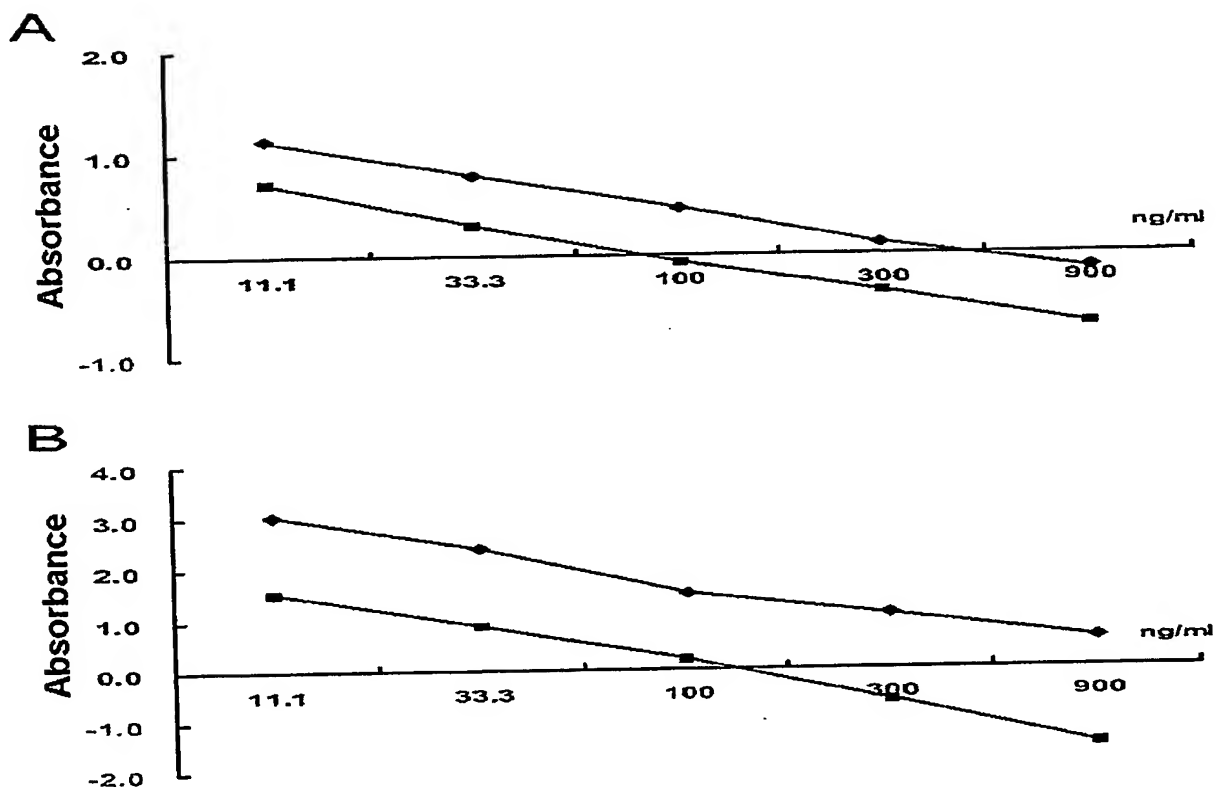


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FIG. 10



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FIG. 11



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FIG. 12

A

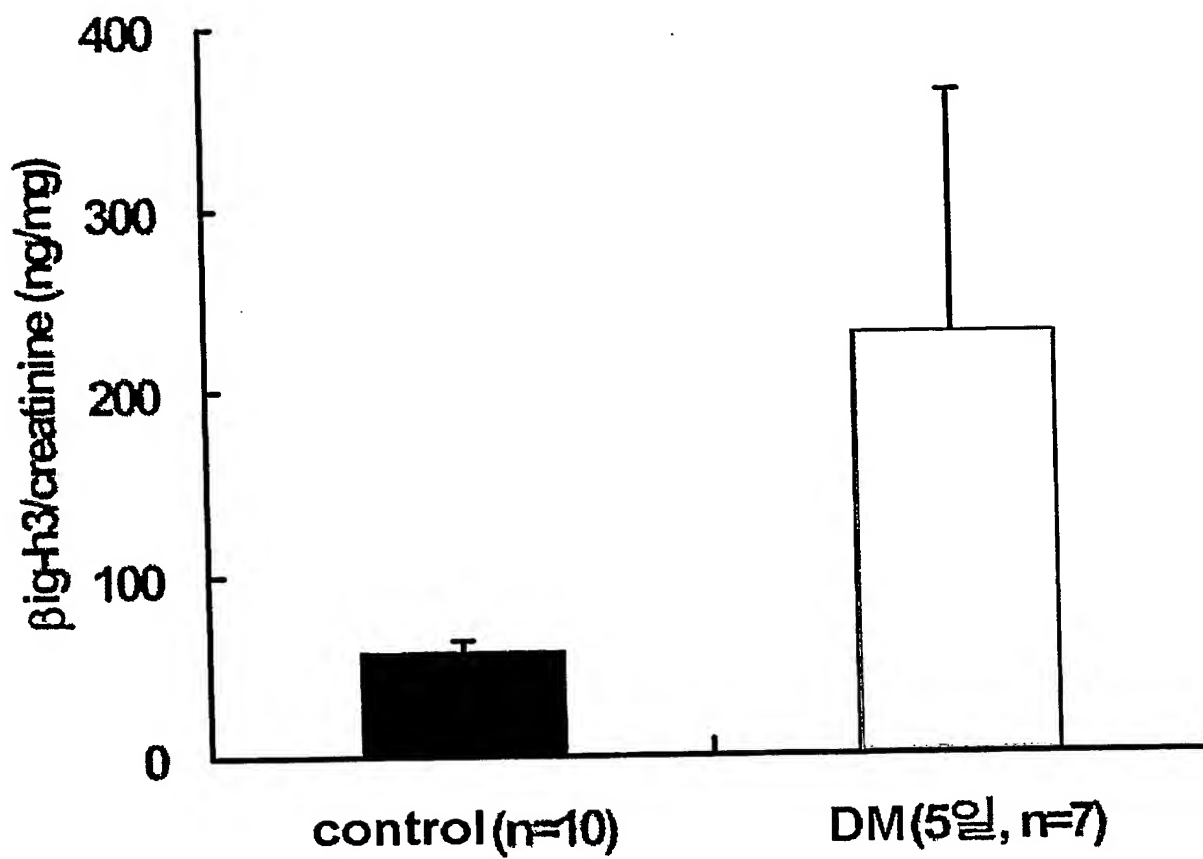


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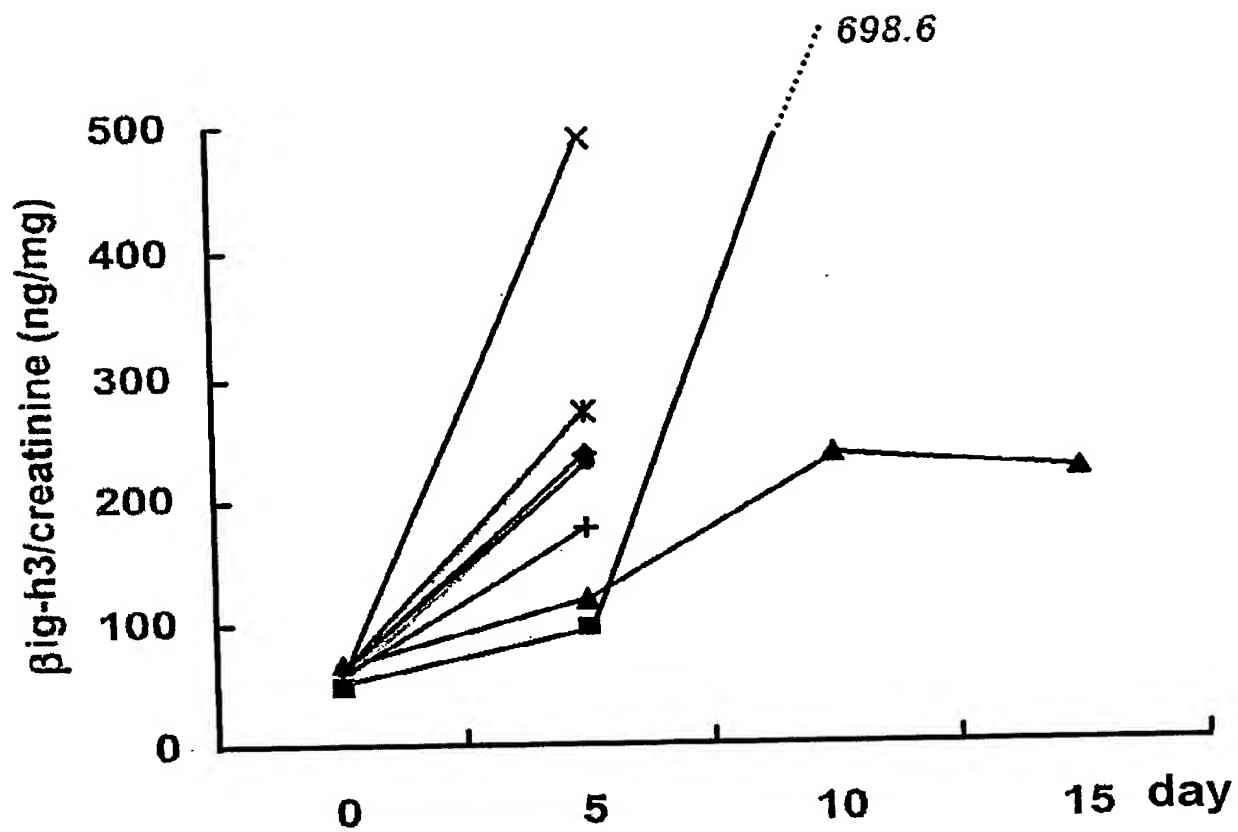
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FIG. 13



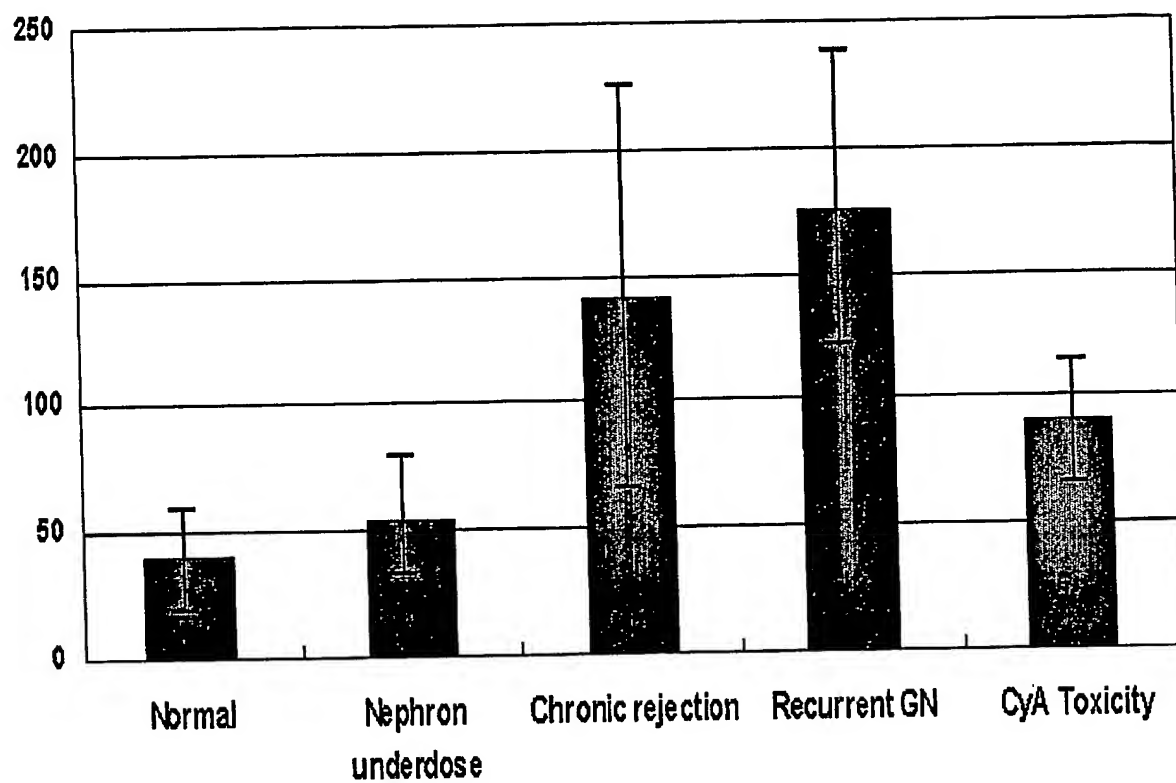
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FIG. 14



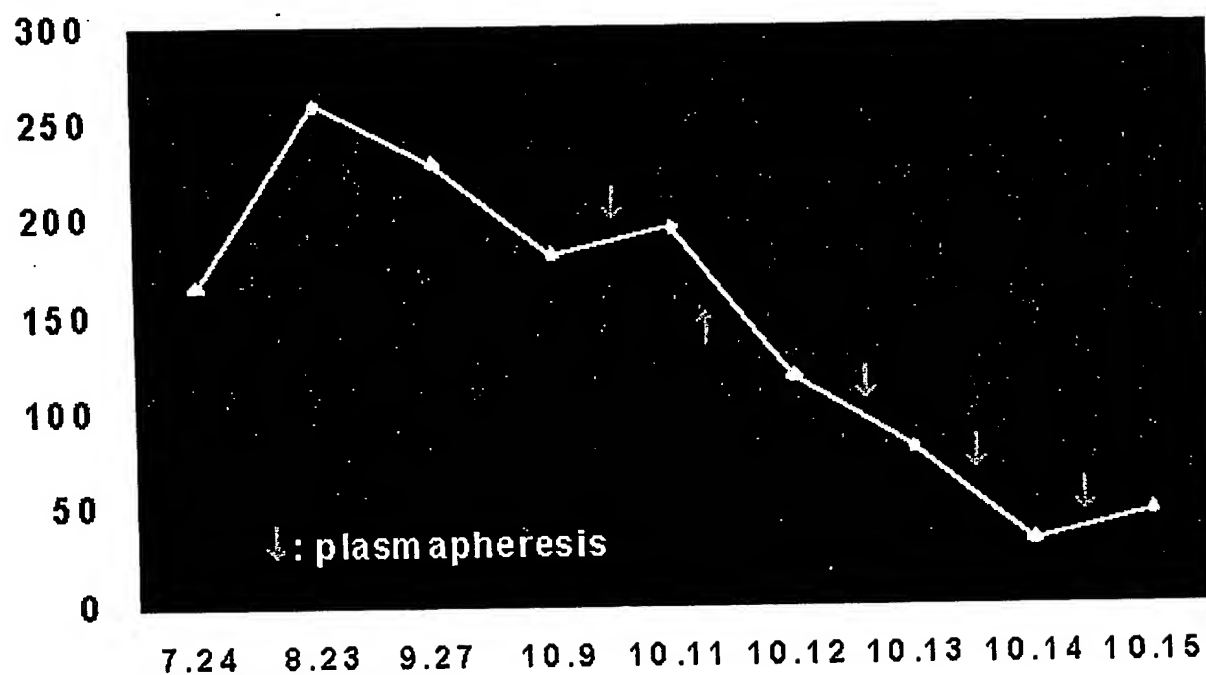
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FIG. 15

 β ig-h3 (ng/mg creatinine)

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FIG. 16

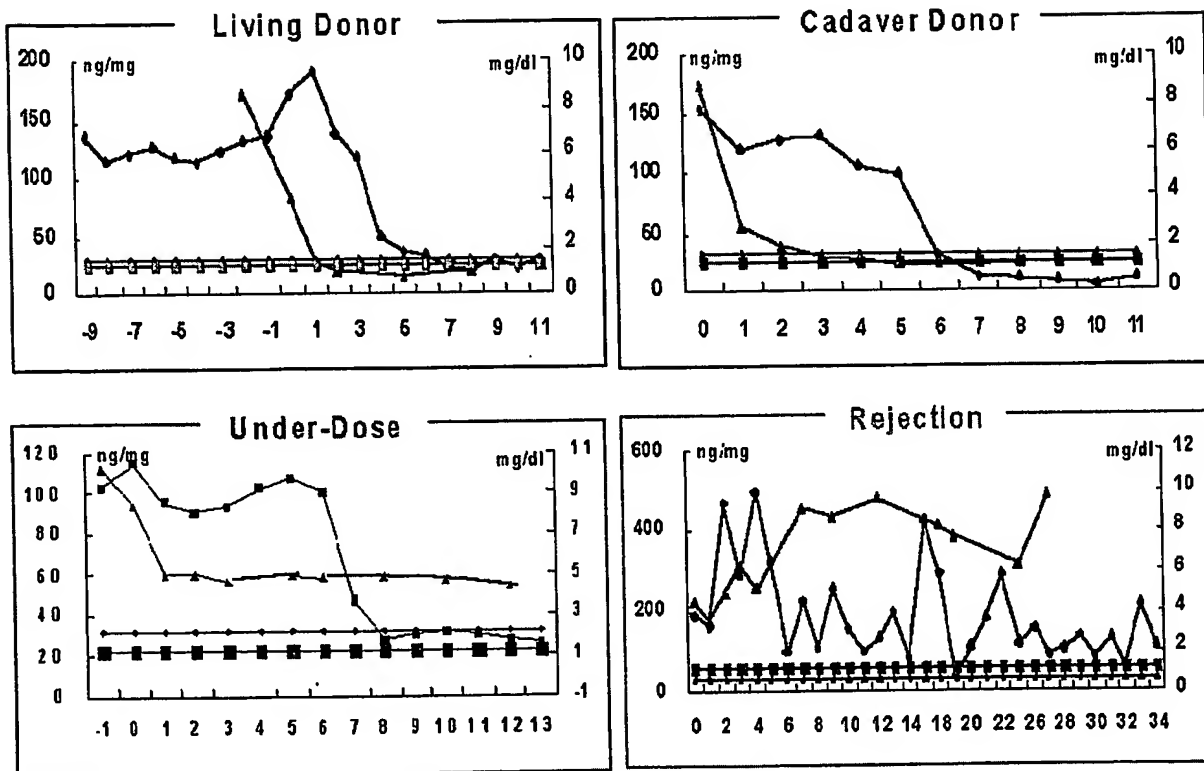
 β lg-h3 (ng/mg creatinine)

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FIG. 17

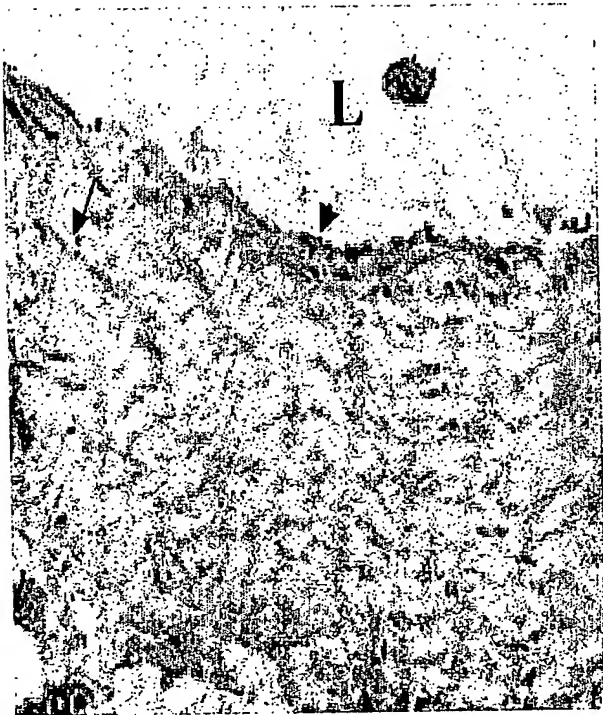
Kidney Transplantation

— pig-h3
— Normal pig-h3
— S.creatinine
— Normal S.creatinine

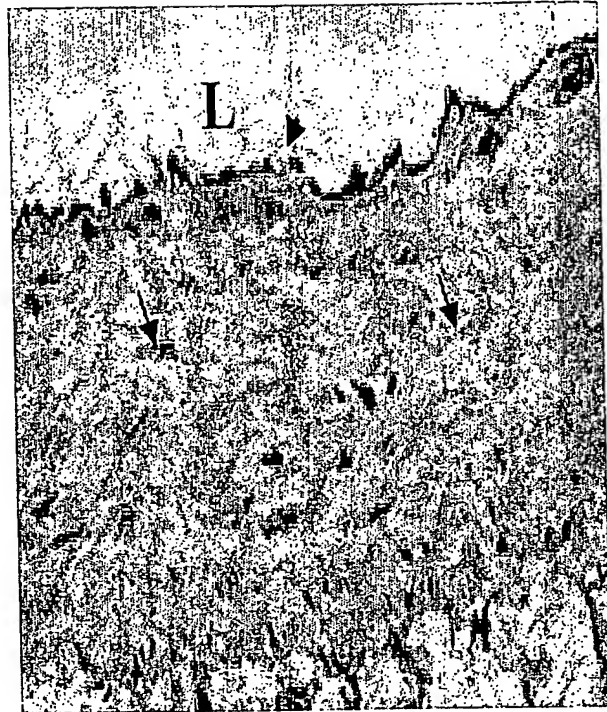


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FIG. 18

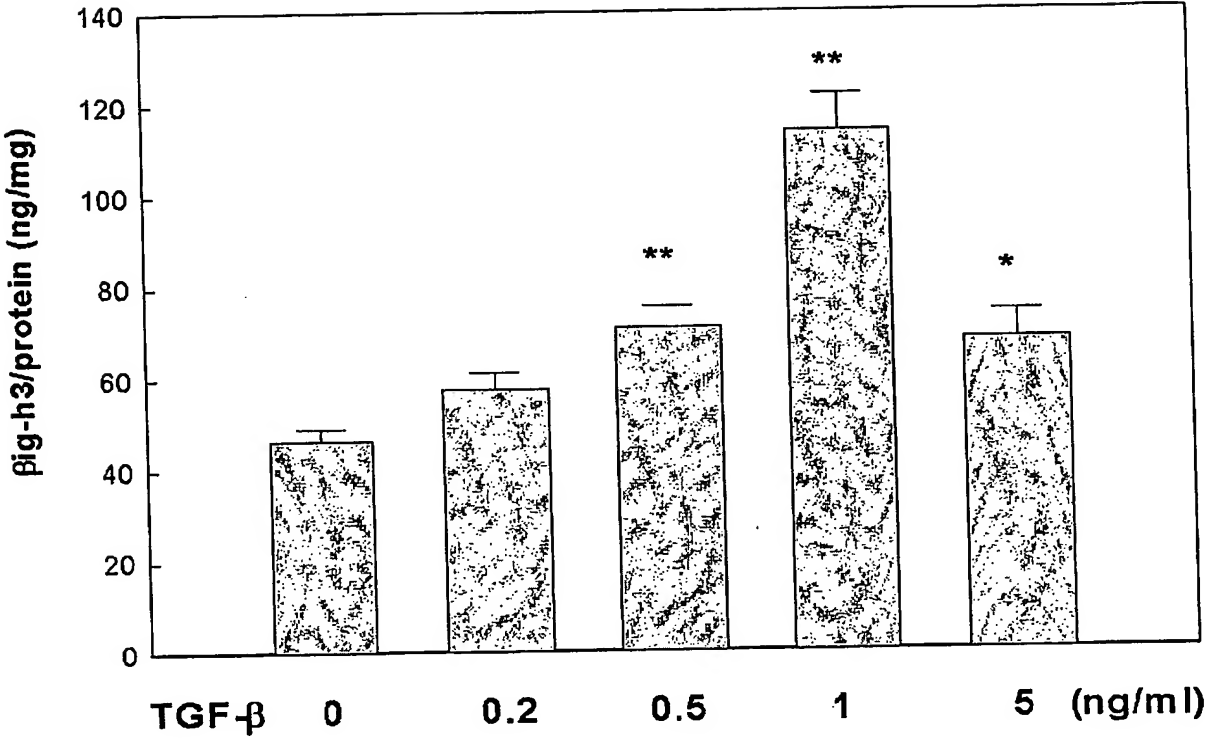


A. Normal tissue (x200)



B. Damaged tissue (x200)

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FIG. 19



SEQUENCE LISTING

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aacaatgtag tgagtgtcaa taaggagcct gttgccgaaa ccgacatcat ggccacaaaac      1800
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<210>      5
<211>     609
<212>      PRT
<213>      Mouse Intracisternal A-particle

<220>
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<222>      (1)..(609)
<223>      23 to 641 amino acid sequence of mouse

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<400>      5
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Leu Arg Gly Arg Gln His Gly Pro Asn Val Cys Ala Val Gln Lys Val
      20              25              30

Ile Gly Thr Asn Arg Lys Tyr Phe Thr Asn Cys Lys Gln Trp Tyr Gln
      35              40              45

Arg Lys Ile Cys Gly Lys Ser Thr Val Ile Ser Tyr Glu Cys Cys Pro
      50              55              60

Gly Tyr Glu Lys Val Pro Gly Glu Lys Gly Cys Pro Ala Ala Leu Pro
      65              70              75              80

Leu Ser Asn Leu Tyr Glu Thr Leu Gly Val Val Gly Ser Thr Thr Thr
      85              90              95

Gln Leu Tyr Thr Asp Arg Thr Glu Lys Leu Arg Pro Glu Met Glu Gly

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100	105	110
Pro Gly Ser Phe Thr Ile	Phe Ala Pro Ser Asn Glu Ala Trp Ala Ser	
115	120	125
Leu Pro Ala Glu Val Leu Asp Ser Leu Val Ser Asn Val Asn Ile Glu		
130	135	140
Leu Leu Asn Ala Leu Arg Tyr His Met Val Gly Arg Arg Val Leu Thr		
145	150	155
Asp Glu Leu Lys His Gly Met Thr Leu Thr Ser Met Tyr Gln Asn Ser		
165	170	175
Asn Ile Gln Ile His His Tyr Pro Asn Gly Ile Val Thr Val Asn Cys		
180	185	190
Ala Arg Leu Leu Lys Ala Asp His His Ala Thr Asn Gly Val Val His		
195	200	205
Leu Ile Asp Lys Val Ile Ser Thr Ile Thr Asn Asn Ile Gln Gln Ile		
210	215	220
Ile Glu Ile Glu Asp Thr Phe Glu Thr Leu Arg Ala Ala Val Ala Ala		
225	230	235
Ser Gly Leu Asn Thr Met Leu Glu Gly Asn Gly Gln Tyr Thr Leu Leu		
245	250	255
Ala Pro Thr Asn Glu Ala Phe Glu Lys Ile Pro Ser Glu Thr Leu Asn		
260	265	270
Arg Ile Leu Gly Asp Pro Glu Ala Leu Arg Asp Leu Leu Asn Asn His		
275	280	285
Ile Leu Lys Ser Ala Met Cys Ala Glu Ala Ile Val Ala Gly Leu Ser		
290	295	300
Val Glu Thr Leu Glu Gly Thr Thr Leu Glu Val Gly Cys Ser Gly Asp		
305	310	315
Met Leu Thr Ile Asn Gly Lys Ala Ile Ile Ser Asn Lys Asp Ile Leu		
325	330	335
Ala Thr Asn Gly Val Ile His Tyr Ile Asp Glu Leu Leu Ile Pro Asp		
340	345	350

Ser Ala Lys Thr Leu Phe Glu Leu Ala Ala Glu Ser Asp Val Ser Thr
 355 360 365
 Ala Ile Asp Leu Phe Arg Gln Ala Gly Leu Gly Asn His Leu Ser Gly
 370 375 380
 Ser Glu Arg Leu Thr Leu Leu Ala Pro Leu Asn Ser Val Phe Lys Asp
 385 390 395 400
 Gly Thr Pro Pro Ile Asp Ala His Thr Arg Asn Leu Leu Arg Asn His
 405 410 415
 Ile Ile Lys Asp Gln Leu Ala Ser Lys Tyr Leu Tyr His Gly Gln Thr
 420 425 430
 Leu Glu Thr Leu Gly Gly Lys Lys Leu Arg Val Phe Val Tyr Arg Asn
 435 440 445
 Ser Leu Cys Ile Glu Asn Ser Cys Ile Ala Ala His Asp Lys Arg Gly
 450 455 460
 Arg Tyr Gly Thr Leu Phe Thr Met Asp Arg Val Leu Thr Pro Pro Met
 465 470 475 480
 Gly Thr Val Met Asp Val Leu Lys Gly Asp Asn Arg Phe Ser Met Leu
 485 490 495
 Val Ala Ala Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg Glu
 500 505 510
 Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala Leu
 515 520 525
 Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu Ala
 530 535 540
 Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly Gly
 545 550 555 560
 Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu Glu
 565 570 575
 Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val Ala
 580 585 590
 Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr Asn
 595 600 605

Val

<210> 6
 <211> 391
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> β ig-h3 D-IV

<400> 6
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 tgtcctgaag ggagacaatc gtttttagcat gctgggtggc gccatccagt ctgcaggact 120
 catggagatc ctcaaccggg aaggggtcta cactgttttt gctcccacca atgaagcggt 180
 ccaagccatg cctccagaag aactgaacaa actcctggca aatgccaagg aacttaccaa 240
 catcctgaag taccacattg gtgatgaaat cctgggttagc ggaggcatcg gggccctggt 300
 gcggctgaag tctctccaag gggacaaaact ggaagtcagc tcgaaaaaca atgtagttag 360
 tgtcaataag gagcctgttg ccgaaaccga c 391

<210> 7
 <211> 140
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> β ig-h3 D-IV(1X) amino acid sequence

<400> 7
 Leu Thr Pro Pro Met Gly Thr Val Met Asp Val Leu Lys Gly Asp Asn
 1 5 10 15
 Arg Phe Ser Met Leu Val Ala Ala Ile Gln Ser Ala Gly Leu Thr Glu
 20 25 30
 Thr Leu Asn Arg Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu

35 40 45
 Ala Phe Arg Ala Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp
 50 55 60
 Ala Lys Glu Leu Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile
 65 70 75 80
 Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln
 85 90 95
 Gly Asp Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn
 100 105 110
 Lys Glu Pro Val Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val
 115 120 125
 His Val Ile Thr Asn Val Leu Gln Pro Pro Ala Asn
 130 135 140

 <210> 8
 <211> 280
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> β ig-h3 D-IV(2X) amino acid sequence

 <400> 8
 Leu Thr Pro Pro Met Gly Thr Val Met Asp Val Leu Lys Gly Asp Asn
 1 5 10 15
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 20 25 30
 Thr Leu Asn Arg Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu
 35 40 45
 Ala Phe Arg Ala Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp
 50 55 60
 Ala Lys Glu Leu Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile
 65 70 75 80
 Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln

85	90	95
Gly Asp Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn		
100	105	110
Lys Glu Pro Val Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val		
115	120	125
His Val Ile Thr Asn Val Leu Gln Pro Pro Ala Asn Leu Thr Pro Pro		
130	135	140
Met Gly Thr Val Met Asp Val Leu Lys Gly Asp Asn Arg Phe Ser Met		
145	150	155
Leu Val Ala Ala Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg		
165	170	175
Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala		
180	185	190
Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu		
195	200	205
Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly		
210	215	220
Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu		
225	230	235
Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val		
245	250	255
Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr		
260	265	270
Asn Val Leu Gln Pro Pro Ala Asn		
275	280	

<210> 9
 <211> 420
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> β ig-h3 D-IV(3X) amino acid sequence

<400> 9
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 1 5 10 15
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 20 25 30
 Thr Leu Asn Arg Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu
 35 40 45
 Ala Phe Arg Ala Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp
 50 55 60
 Ala Lys Glu Leu Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile
 65 70 75 80
 Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln
 85 90 95
 Gly Asp Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn
 100 105 110
 Lys Glu Pro Val Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val
 115 120 125
 His Val Ile Thr Asn Val Leu Gln Pro Pro Ala Asn Leu Thr Pro Pro
 130 135 140
 Met Gly Thr Val Met Asp Val Leu Lys Gly Asp Asn Arg Phe Ser Met
 145 150 155 160
 Leu Val Ala Ala Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg
 165 170 175
 Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala
 180 185 190
 Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu
 195 200 205
 Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly
 210 215 220
 Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu
 225 230 235 240

Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val
 245 250 255

Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr
 260 265 270

Asn Val Leu Gln Pro Pro Ala Asn Leu Thr Pro Pro Met Gly Thr Val
 275 280 285

Met Asp Val Leu Lys Gly Asp Asn Arg Phe Ser Met Leu Val Ala Ala
 290 295 300

Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg Glu Gly Val Tyr
 305 310 315 320

Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala Leu Pro Pro Arg
 325 330 335

Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu Ala Asn Ile Leu
 340 345 350

Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly Gly Ile Gly Ala
 355 360 365

Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu Glu Val Ser Leu
 370 375 380

Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val Ala Glu Pro Asp
 385 390 395 400

Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr Asn Val Leu Gln
 405 410 415

Pro Pro Ala Asn
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<210> 10
 <211> 560
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> β ig-h3 D-IV(4X) amino acid sequence

<400> 10

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 1 5 10 15
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 20 25 30
 Thr Leu Asn Arg Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu
 35 40 45
 Ala Phe Arg Ala Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp
 50 55 60
 Ala Lys Glu Leu Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile
 65 70 75 80
 Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln
 85 90 95
 Gly Asp Lys Leu Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn
 100 105 110
 Lys Glu Pro Val Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val
 115 120 125
 His Val Ile Thr Asn Val Leu Gln Pro Pro Ala Asn Leu Thr Pro Pro
 130 135 140
 Met Gly Thr Val Met Asp Val Leu Lys Gly Asp Asn Arg Phe Ser Met
 145 150 155 160
 Leu Val Ala Ala Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg
 165 170 175
 Glu Gly Val Tyr Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala
 180 185 190
 Leu Pro Pro Arg Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu
 195 200 205
 Ala Asn Ile Leu Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly
 210 215 220
 Gly Ile Gly Ala Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu
 225 230 235 240
 Glu Val Ser Leu Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val
 245 250 255

Ala Glu Pro Asp Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr
 260 265 270

Asn Val Leu Gln Pro Pro Ala Asn Leu Thr Pro Pro Met Gly Thr Val
 275 280 285

Met Asp Val Leu Lys Gly Asp Asn Arg Phe Ser Met Leu Val Ala Ala
 290 295 300

Ile Gln Ser Ala Gly Leu Thr Glu Thr Leu Asn Arg Glu Gly Val Tyr
 305 310 315 320

Thr Val Phe Ala Pro Thr Asn Glu Ala Phe Arg Ala Leu Pro Pro Arg
 325 330 335

Glu Arg Ser Arg Leu Leu Gly Asp Ala Lys Glu Leu Ala Asn Ile Leu
 340 345 350

Lys Tyr His Ile Gly Asp Glu Ile Leu Val Ser Gly Gly Ile Gly Ala
 355 360 365

Leu Val Arg Leu Lys Ser Leu Gln Gly Asp Lys Leu Glu Val Ser Leu
 370 375 380

Lys Asn Asn Val Val Ser Val Asn Lys Glu Pro Val Ala Glu Pro Asp
 385 390 395 400

Ile Met Ala Thr Asn Gly Val Val His Val Ile Thr Asn Val Leu Gln
 405 410 415

Pro Pro Ala Asn Leu Thr Pro Pro Met Gly Thr Val Met Asp Val Leu
 420 425 430

Lys Gly Asp Asn Arg Phe Ser Met Leu Val Ala Ala Ile Gln Ser Ala
 435 440 445

Gly Leu Thr Glu Thr Leu Asn Arg Glu Gly Val Tyr Thr Val Phe Ala
 450 455 460

Pro Thr Asn Glu Ala Phe Arg Ala Leu Pro Pro Arg Glu Arg Ser Arg
 465 470 475 480


Leu Leu Gly Asp Ala Lys Glu Leu Ala Asn Ile Leu Lys Tyr His Ile
 485 490 495

Gly Asp Glu Ile Leu Val Ser Gly Gly Ile Gly Ala Leu Val Arg Leu

500	505	510
Lys Ser Leu Gln Gly Asp	Lys Leu Glu Val Ser Leu Lys Asn Asn Val	
515	520	525
Val Ser Val Asn Lys Glu Pro Val Ala Glu Pro Asp Ile Met Ala Thr		
530	535	540
Asn Gly Val Val His Val Ile Thr Asn Val Leu Gln Pro Pro Ala Asn		
545	550	555 560

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/01975

A. CLASSIFICATION OF SUBJECT MATTER IPC7 G01N 33/68 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC7 G01N 33/53, C07K 15/00, C12N 15/09, A61K 9/06 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean Patents and applications for inventions since 1975, Korean Utility models and application for Utility models since 1975. Japanese Utility models and applications for Utility models since 1975. Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) NCBI, MEDLINE, eKIPASS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/01102 A (Advanced Tissue Sciences) 18. 01. 1996 - see the whole document -	1-16
Y	EP 555,989 A (Purchio, Anthony F) 18. 08.1993 - example 1, 2	1-16
Y	US 5,714,588 A (Advanced Tissue Sciences) 03. 02. 1998 - see the whole document -	1-16
Y	US 5,444,164 A (Bristol-Myers Squibb Company) 22.88. 1995 - see the abstract, example -	1-16
Y	US 5,599,788 A (Advanced Tissue Sciences) 04. 02. 1997 -see the whole document -	1-16
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 27 FEBRUARY 2003 (27.02.2003)		Date of mailing of the international search report 27 FEBRUARY 2003 (27.02.2003)
Name and mailing address of the ISA/KR  Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140		Authorized officer JOO, Young Sik Telephone No. 82-42-481-5995 